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Significance of pesticide emission modelling for assessing ecotoxicity impacts of agricultural systems

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SETAC Europe

Helsinki 2019

SETAC Europe 29th Annual Meeting

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Abstract Book

One Environment. One Health.
Sustainable Societies.

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Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)

ABSTRACT BOOK
SETAC Europe 29th Annual Meeting

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This book compiles the abstracts from the platform and poster session presentations at the 29th Annual Meeting of the Society of Environmental Toxicology and Chemistry- Europe (SETAC Europe), conducted at the Messukeskus Helsinki, Expo and Convention Centre, Helsinki, Finland, from 26–30 May 2019.
The abstracts are reproduced as submitted by the author and accepted by the Scientific Committee. They appear in order of abstract code and alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is underlined.

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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY
In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, managers, engineers or others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void, and quickly saw dynamic growth in the Society's membership, meeting attendance and publications.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers of the World Council and Geographic Unit Boards of

Directors and Councils, and in the composition of committees and other society activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality Through Science®, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Therefore, SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

SETAC's growth is reflected in the founding of geographic units around the world. SETAC Europe was established in 1989 as an independent organisation, followed by SETAC Asia-Pacific in 1997 and SETAC Latin America in 1999. In 2002, the four existing organisations joined together under the governance of the SETAC World Council. SETAC Africa is the most recent geographic unit, which was adopted in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters and branches have emerged in a number of countries.

SETAC publishes two journals: *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM). Environmental Toxicology and Chemistry is dedicated to furthering scientific knowledge and disseminating information on environmental toxicology and chemistry, including the application of these sciences to risk assessment. Integrated Environmental Assessment and Management focuses on the application of science in environmental decision-making, regulation, and management, including aspects of policy and law, and the development of scientifically sound approaches to environmental problem solving. Together, these journals provide a forum for professionals in academia, business, government, and other segments of society involved in the use, protection, and management of the environment for the enhancement of ecological health and human welfare.

SETAC books provide timely in-depth reviews and critical appraisals on scientific subjects relevant to understanding a wide range of contemporary topics pertaining to the environment. These include any aspect of environmental chemistry, toxicology, risk assessment, risk management, or environmental policy.

SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1992, and in Brussels, Belgium, established in 1993.

Keynote Abstracts

Keynote Sunday

One Health, Persistent Organic Pollutants and the Arctic

Cynthia A. de Wit, Department of Environmental Science and Analytical Chemistry, Stockholm University, Stockholm, Sweden

The One Health concept is based on understanding how human, wildlife and ecosystem/planetary health are interconnected and the Arctic has long served as an early warning system in this regard. Persistent organic pollutants will be used to illustrate this, for example, the early discovery of PCBs in the Arctic in the 1970s, and subsequent studies of PCBs in wildlife and humans in the Canadian Arctic in the 1980s. The very high levels found in top predators and some indigenous populations in what was supposed to be a pristine environment was devastating news. Indigenous peoples' traditional foods, crucial for their health, society and culture were now highly contaminated. These and other findings led the eight Arctic countries to call for an assessment of the Arctic environment. A report on POPs, other contaminants and human health in the Arctic environment was presented by the Arctic Monitoring and Assessment Programme in 1998.The science presented led to new understanding of pathways and processes for POPs into and within the Arctic, including biomagnification in food webs and humans, and effects of exposure. It illustrated succinctly how interconnected the Arctic was to the rest of the world, and was a driving factor in development of regional and global POPs regulation, such as the Stockholm Convention. Further protection of the Arctic from new chemicals is thus crucial to protecting humans, wildlife and ecosystems globally.

Keynote Monday

Can One Environment, One Health and Many Chemicals be Sustainable?

Bjorn Hansen, European Chemicals Agency (ECHA), Finland

With the adoption in 2015 of the Sustainable Development Goals the world embarked on an ambitious journey to obtain a sustainable future by 2030. Chemicals management has a specific target within Sustainable Development Goal 14 is "By 2020, achieve the environmentally sound management of chemicals and all wastes throughout their life cycle, in accordance with agreed international frameworks, and significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment". The EU is settings its chemicals and waste policies, within the EU and internationally, to meet this goal.

The EU has also set an ambitious goal of moving towards a circular economy. Achieving circularity requires significant advances in product design, in material development, in collection and recycling/recovery. At the core are materials –as it is the material which gives the product its desired functionality and enables recycling or recovery. For those working in the chemicals area a material is a mixture.

To achieve the sustainable development goals in an EU moving towards a circular economy, requires innovation in chemistry to develop the chemicals which make up circular materials and which minimize the adverse impacts on human health and the environment. There is though much which needs to be put in place to achieve this: from chemicals and waste legislation, from research and industry. But meeting the challenge is a necessity if we want to protect our one environment, provide protection for all citizens and ensure that chemicals can be used to maintain our wellbeing!

Keynote Tuesday

Environmental Pollutants and Health

Jorma Toppari, University of Turku, Finland

Environmental effects on health are in many cases preventable in contrast to genetic impact. Industrial chemicals have been produced only during the last 150 years and the production has multiplied during our time. Unfortunately many of the good products turned out to be bad pollutants affecting our health. Thanks to the Stockholm convention, the amount of persistent organic pollutants is declining, and now the main attention is on less persistent compounds that interfere with physiology, including endocrine and immune systems, central nervous system, and most importantly, their development. The incidence of non-communicable diseases in increasing and many environmental chemicals are associated with their pathophysiology. Endocrine disrupting chemicals can contribute to reproductive disorders and cognitive development by inhibiting hormone production and action at critical developmental phases. Anti-androgenic chemicals cause genital malformations and poor semen quality experimentally and most likely in humans, too. Anti-thyroid chemicals can cause severe adverse effects in central nervous system in fetuses and infants. Currently the greatest problem is the lack of knowledge about the health effects of the majority of chemicals in use.

Keynote Wednesday

Innovation Where Everyone Wins: A Call for a New Dialogue in Environmental Toxicology, Chemistry and Risk Assessment

Juan Gonzalez Valero, Syngenta, Switzerland

Since the early days in environmental sciences we have constantly increased our understanding off the impacts of human activities and technologies on the environment. We are experts in exploring risks and it is only logical that we focus our effort on avoiding the risk from those activities. We know that we don't have all the answers and have to apply precaution. But we shouldn't confuse precaution with avoidance. Avoiding innovation means avoiding progress, and without progress there will be no better outcome: not for humans and not for nature. As environmental scientist we are in a dilemma. The possible trade-offs are complex and require a different form of dialogue that pushes us all out of our comfort zone. A common reaction is to push decisions to the political level with detrimental effects on safeguarding and improving the quality and credibility of science in environmental toxicology and chemistry. We should rather shape a better and more inclusive science dialogue that focuses on the multiple facets of innovation benefits and risks based on reproducible science, unbiased data, analysis, interpretation and disclosure.

Platform Abstracts

Micro(Nano)plastic Pollution: Tackling the Plastic Problem by Identifying Sources, Investigating Fate and Novel Approaches (I)

1

Tracking the origin of fiber micro-fragments in textiles and understanding the release mechanism during washing

Y. Cai, T. Yang, Empa Swiss Federal Laboratories for Materials Science and Technology; D.M. Mitrano, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering; M. Heuberger, Empa Swiss Federal Laboratories for Materials Science and Technology / Laboratory for Advanced Fibers; R. Hufenus, Empa Swiss Federal Laboratories for Materials Science and Technology; B. Nowack, Empa Swiss Federal Laboratories for Materials Science and Technology / Technology and Society Lab

As an important source of microplastics into the environment, many efforts have been made to understand the release (mechanisms) of fiber micro-fragments (FMFs) from synthetic textiles during washing. However, the factors of the materials, finishing processes and washing conditions controlling these releases still remain unclear. The aim of this work was to investigate the formation of FMFs during fiber and fabric manufacturing and the mechanism controlling the FMF release during washing. An extraction method was developed to investigate the FMFs formed through the production chain of textiles. Several representative polyester products along the production line including a sliver, three types of yarns and eleven kinds of fabrics were collected. The extraction was performed by sequential ultra-sonication and the released fibers were collected on filter. In this way, we developed a method to decouple the release of FMFs that were already in the textile sample from the potential formation of new FMFs from mechanical stress during washing. The FMFs were imaged and analyzed to obtain the length and the number. The second experiment was performed with a gyrowash machine and a standard wash cycle mainly based on an ISO washing standard. Additional experiments were performed to understand the influence by washing time, mechanical stress and concentration of detergents. The extraction experiment showed that after three to nine 10-minute sonication steps, the release of FMFs dropped to almost zero, indicating that sonication less than 90 minutes did not produce FMFs but was rather releasing what is already present inside the fiber or fabric. The release was affected by the type of fiber (e.g. staple fiber), the fabric structure and the cutting method. Those fabrics which showed higher releases in the extraction experiment also shed more FMFs during washing. We observed a decrease of the released FMFs with the number of washing cycles, indicating that formation of FMF during washing is very low. The number of FMFs extracted by ultrasound and the FMF release during washing was in the same magnitude and demonstrated a linear correlation suggesting that the washing process is likely only shedding FMFs embedded in the fabric instead of producing new ones. The results of this work will establish a deeper understanding of the origin of the FMF released from textiles and may form the basis for engineering options to reduce FMFs in new fabrics being produced.

2

PLASTIC-Seine research project: Microplastic Flux and Impact on biota in the Seine estuary

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Microplastics (MP) pollution is of emerging concern. Although numerous studies were recently carried out on freshwater and biota contamination, studies focused on estuary ecosystems are scarce. Estuaries can indeed play a significant role in the transport and transfer of MP to the ocean and the estuarine hydrodynamics and salinity gradient can greatly affect both MP distribution and concentrations, as well as the occurrence and levels in biotic compartments. In this context, the Plastic-Seine project (2017-2020) involving six French laboratories was launched to study the occurrence and levels of MP in all compartments of the ecosystem of the Seine River estuary (France). The objectives are: i) to study the occurrence and levels of MP in the Seine estuary for abiotic compartments (water column and sediment), ii) to study the ingestion and levels of MP for seven emblematic species of the Seine estuary, i.e, the worm *Hediste diversicolor*, the copepod *Eurytemora affinis*, the mussel *Mytulis edulis*, the prawn *Palaemon longirostris* and 3 fish species *Platichthys flesus*, *Solea solea* and *Dicentrarchus labrax* and iii) to assess the potential impacts of MP exposure on biology traits, physiology and behavior of three species (*Hediste diversicolor*, *Eurytemora affinis* and *Solea solea*). Preliminary and main results are here presented and discussed. First results on abiotic compartements highlighted the presence of fibers and fragements in both the water colonn and sediments. Particles exhibited different forms depending on their origin and/or fragmentation. Most of particles were inferior to 1 mm and a high proportion of fragments and films was higlighted. Slight MP distribution changes were also observed between surface water and subsurface water. For the seven species, MP including fibers and fragements were found. The others analyses are in progress. For all species, a high variabilities between species and individuals was highligted. High presence of fragments were noticed for seabass and worms in comparison to other species. In the case of prawns, a large amount of MP and especially of fibers were also observed on the cuticle surface. Futher studies will investigate the potential impacts of MP exposure on biology traits, physiology, and behavior of three estuarine species and the MP trophic transfer to common sole's food chain.

3

A comprehensive investigation of microplastic contamination in Lake Mjøsa, Norway's largest lake

A. Lusher, NIVA Norwegian Institute of Water Research / Environmental Contaminants; N. Buenaventura, NIVA Norwegian Institute for Water Research / Environmental Contaminants; D. Eidsvoll, Norwegian Institute for Water Research NIVA / Ecotoxicology and Risk Assessment; J. Thrane, NIVA Norwegian Institute for Water Research / Pelagic freshwater ecology; A. Økelsrud, NIVA Norwegian Institute for Water Research; M. Jartun, NIVA Norwegian Institute for Water Research / Environmental Contaminants

Plastic is a ubiquitous contaminant in the environment, this includes microplastics (< 1 mm) which have been found globally. Some microplastics are released directly into the environment whereas others break down from larger plastic items because of environmental processes. The widespread presence of microplastics in the environment highlights the need to understand the sources for microplastics and the consequences of this contamination. In this current project, our aim was to contribute to knowledge concerning microplastic contamination in freshwater environments. To do so, an assessment of microplastic contamination in the largest lake in Norway, Lake Mjøsa, was carried out looking at sediments, biota and water samples. Microplastics were identified in all core slices (0-1cm, 1-2cm, 2-3cm) across twenty stations with varying anthropogenic sources of input. Polymer composition varied between sites and core depth. For example, polyamide was less abundant in deeper core slices, polycarbonate was found at three sites and polyvinyl chloride was only found at one site. In addition, microplastics were identified in 9/12 historical plankton samples dated 1973 to 2017. Only a single duck mussel was found to contain an orange synthetic rubber fragment (339 x 233 μm). In forthcoming months, the deeper core slices (3-4 cm, 7-8cm) will be assessed and compared to the currently complied data. This study will also be essential for sealing the knowledge gaps in microplastic studies, which will enable this field to be further developed and provide a better understanding of the effects and impacts of microplastics in the environment. The results from this study will form the basis for a recommendation for further monitoring of microplastics in Lake Mjøsa, and in freshwater in general.

4

Assessing the relevance of wastewater and agricultural runoff as microplastic sources for freshwater ecosystems: A case study in central Spain

T.C. Schell, IMDEA Water Institute / Ecotoxicology; R. Hurley, NIVA - Norwegian Institute for Water Research; A. Rico, IMDEA Water Institute / Aquatic Ecotoxicology; L. Nizzetto, Norwegian Institute for Water Research NIVA; M. Vighi, IMDEA Water Institute / Earth and Environmental Sciences

Microplastics (MPs) in wastewater are mostly retained in the sludge during wastewater treatment. Despite the high trapping efficiency of wastewater treatment plants (WWTPs), treated wastewater can act as a considerable source of MPs if large quantities are discharged. Sludge applied as fertilizer on agricultural fields may act as an additional MP source for freshwater ecosystems if MPs are transported by water runoff. However, no data is indicating the relevance of this environmental pathway. This study aimed to comparatively assess the contribution of WWTP effluents and runoff from agricultural fields as sources of MPs into freshwater ecosystems, and to assess MPs concentrations and composition in streams receiving such pollution sources. The study was conducted in the Henares River Watershed (central Spain). MP concentrations were monitored in treated and untreated wastewater and sludge from five different WWTPs. An experiment was performed to assess the MP concentrations in runoff from agricultural fields. For this, three plots (2 m^2) with comparable soil characteristics but differing sludge applications were prepared: control soil (never treated with sludge), soil treated in the past (in 2013), and soil treated at the start of the experiment (in November 2017). Runoff samples were collected throughout one year. Additionally, the MP concentration was monitored at three different river sites: i) low human impact; ii) agricultural impact; and ii) urban, agricultural and

industrial impact. All water samples were taken by filtering sufficient amounts of water (river or wastewater) through a battery of plankton nets with different mesh size (300, 150, 55, 20 μm). Following MP extraction, the number and polymer composition per sample were assessed. Initial results (based on MPs > 150mm) show that 58% to 99% of MPs are retained during wastewater treatment, but still, up to 400 MPs/m^3 are emitted. The runoff experiment shows that MPs are present and mobilized via runoff water from all plots, but the highest concentration (1.36 MP/L runoff or 14.5 MPs per m^2) was observed in the most recently treated plot. To our knowledge this is the first study evaluating the contribution of sewage sludge to MP freshwater contamination. The results indicate that both, treated wastewater and runoff from sewage sludge treated fields, can act as a MPs source for aquatic ecosystems and should be taken into account in risk assessments performed at the watershed scale.

5

Matrix-adapted methodologies for qualitative and quantitative study of microplastics in marine bivalves and sediment

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About 335 million tons of plastics were produced in 2016 and this production is increasing every year, due to their numerous properties and resulting societal benefits. The distribution of MPs in the environment depends on various inherent factors. The omnipresence of MPs has been demonstrated by scientists in recent years, particularly in marine ecosystems. Sediments are generally recognized as a large contaminated environmental compartment. But fauna is also affected, especially filter-feeder species. The aim of this work was to develop and validate rapid methodologies for assessing the MP contamination in sediment and bivalves (mussels: *Mytilus edulis* and oysters: *Crassostrea gigas*) from the French Atlantic coast (Pays de la Loire region, France). Surface sediments (0 to -5 cm) and bivalves were sampled at three locations and in two seasons: October 2015 and March 2016. Ten replicates of 25 g of sediment, 10 pools of 3 mussels and 10 individual oysters were analyzed per sampling location and season, leading to a global analysis of 60 sediment samples, 180 mussels and 90 oysters. In order to analyze the MPs, rapid protocols were adapted to the matrix and validated using spiked samples for isolation and identification of MPs without visual sorting. Good recoveries were obtained in the validation tests with values ranging from 78 % to 107 % depending on MP types. A total of 10 different types were found with a majority of PP and PE. Average levels ranged from 38 (± 47) to 102 (± 105) MP per kg of dry sediment (N = 10; 250 g). The whole abundance of MPs found in mussels and oysters respectively were about 0.23 (± 0.20) and 0.18 (± 0.16) MP per gram of wet weight soft tissues and about 0.60 (± 0.56) and 2.10 (± 1.71) MP per individual. It exists inter-compartment and interspecific differences on type, size and color of MPs. Globally, more than 80% of MPs were fragments, the remaining were filaments. None of granule or pellet were found. MP color showed high proportion of grey to light-grey. This littoral region adjacent to the Atlantic Ocean gathers significant areas of aquaculture at national level, for which the characterization of MP contamination is of great concern. Therefore, monitoring the presence of MPs in marine areas is currently of high importance as some of the organisms, such as mussels and oysters, are harvested by humans. This work provided the first dataset for this aquaculture area of great socio-economic importance.

6

Microplastic debris in an Italian river: first monitoring results

c. campanale, National Research Council, Water Research Institute; G. Bagnuolo, C. Massarelli, Italian National Research Council; V. Uricchio, Italian National Research Council / Water Research Institute

Plastic and microplastic pollution is one such emerging concern. Plastic pollution in the marine environment is well documented, however, there is a lack of knowledge for freshwater and terrestrial environments in terms of the extent, occurrence and impacts. Herein we report results from a monitoring study aimed to investigate about abundance, type, composition, and potential implications of microplastic in an Italian river (Ofanto river), evaluating at the same time the presence of temporal trends in response to hydrological parameters (flow velocity, water level of river and precipitation). River surface microplastic samples were collected during five monitoring campaigns (February, April, October, December 2017 and May 2018);all of them were taken from the same point located at 6 km from Ofanto river mouth. In order to reduce the spatial variability, microplastics were collected by three surface plankton nets of 333µm mesh size fixed in the middle of the river simultaneously for two different time slot for a total of six replicates for each campaign. Chemical digestion was performed to each sample in order to remove the labile organic matter and extract all the particles. After processing, a visual and chemical characterization has been carried out. Each particle visually identified, was counted, photographed, enumerated, and categorized according to size, color and morphology. Microplastics were found in each net sample for a total of 42,725 items counted, photographed, enumerated and categorized. Microplastic content measured in Ofanto river (expressed as mean value ± dev.st.) ranged from 0.9 ± 0.4 p/m^3 to 12 ± 5 p/m^3 showing values comparable to or greater than those reported in other studies, although there are few other freshwater studies to which to compare. A statistically significant difference in the average microplastic concentrations found in different campaigns of this study, has been observed, suggesting thus a temporal variation in plastic abundances. These differences could be explained by the hydrology of the river that influence, with physical forces the concentrations of particles. Microplastics were found at higher concentrations during wet periods (February 2017 and May 2018) indicating a presumable land-based origin probably from surroundings agricultural areas. The work reported here is the first study showing an Italian river context that provides an initial assessment of the extent and nature of this pollution in Ofanto river.

Effect Modelling for Regulatory Risk Assessment: Current Applications and Future Directions (I)

7

Integrating Semantic Technologies in Environmental RiskAssessment: A Vision

E.B. Myklebust, NIVA - Norwegian Institute for Water Research / Enviromental Data Science; K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment; E. Jimenez-Ruiz, University of Oslo / Department of informatics; Z. Rudjord, R. Wolf, NIVA

Extending the scope of risk assessment models is a long-term goal in ecotoxicological research. However, biological effect data is only available for a few combinations of species–chemical pairs. We aim at designing tools based on semantic technologies to improve risk assessment analysis.\n\nRisk assessment requires the use of different metadata, e.g., from taxonomies, chemicals, or toxicity data. In addition, data collected by researchers in the field or the lab is used as direct input. A partial goal of our work is to effectively use semantic technologies to link these datasets together. These metadata form the basis of a knowledge graph. We add an ontological layer onto the knowledge graph, this includes rules and axioms to either validate or expand the data.\n\nExpanding the data can be done by two approaces, inductive and deductive.\n\nFirst approach considers adding axioms (e.g. ``all Solibacteres is also Bacteria") to the data ontology such that we can use reasoning to increase the available data for the risk assessment.\n\nThe alternative takes a probabilistic approach, using a machine learning model to give a probability of a entity beeing similar to another based on the data. To replace the function of the axioms as an explainer for adding new data, we introduce a new module, that uses the metadata ontology to inform the user about the uncertainty involved in the prediction.\n\nWe develop robust case studies to test system development. We need cases with a complete qualitative performance analysis, compared to previous single compound analysis. A simple such study could include pesticide montoring, NCBI taxonomy, and effects data in the for of ECOTOX.\n\nThis approach will yield new insight by combining and inferring from available data and subsequently improve the quality of risk assessment.

8

QSAR models in ecotoxicology: why such lack of faith?

P. Bicherel, F. Sahigara, KREATiS

Quantitative Structure-Activity Relationship models (QSARs) is one of the powerful alternatives to experimental tests. These statistical models based on semi-empirical approach are used to predict chemical properties based on their molecular structure. These tools are supported by the 3R principle promoting the Refinement, the Reduction and the Replacement of the experimentation performed on animals. Since decades, several QSARs have been developed to predict ecotoxicological and physico-chemical properties. Today many models are available through free software like EPISUITE, ECOSAR, T.E.S.T., VEGA etc. Despite their clear advantages, they have not been massively used as alternatives for REACH registration dossiers between 2009 and 2016. For the endpoints requested for Annexes VII-X and needing to test on vertebrates, QSARs were never the preferred option compared to in vitro studies, Read-Across or Weight of evidence strategies except for the bioaccumulation. In average they represent less than 5% of the endpoint study records. This striking under-use of the QSAR models has been investigated. Several free QSARs have been analysed through the 5 criteria of QSAR validation settled by the OECD among other criteria in order to highlight the main reasons of their feeble use. The principal causes identified are: the lack of transparency about the scientific method on which relies the modelling, the access of the statistics ensuring the performances of the model, the definition of the applicability and its systematic analysis for each prediction and finally the accuracy of the result and the measure of its uncertainty. Good and transparent modelling practises combined to the assessment of the prediction are essential to give credibility to QSARs outcomes.

9

Application of TKTD models in aquatic ERA for pesticides

T. Brock, Wageningen Environmental Research / Environmental Risk Assessment Team; M. Arena, EFSA - European Food Safety Authority / Pesticides; N.

Cedergreen, University of Copenhagen / Plant and Environmental Sciences; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; S. Duquesne, German Environment Agency; A. Focks, Wageningen Environmental Research / Environmental Risk Assessment Team; A. Ippolito, EFSA - European Food Safety Authority / Pesticides Unit; M. Klein, Fraunhofer IME; M. Reed, HSE; I. Teodorovic, University of Novi Sad / Department of Biology and Ecology, Laboratory of Ecotoxicology (LECOTOX)

In 2018, the Panel on Pant Protection Products and their Residues (PPR) of the European Food Safety Authority (EFSA) published a scientific opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) models in prospective environmetal risk assessment (ERA) for pesticides and aquatic organisms (*EFSA Journal 2018;16(8):5377*). In this scientific opinion it is concluded that GUTS models (General Unified Threshold models of Survival; *Jager et al. 2011. Environmental Science and Technology, 45, 2529–2540*) are sufficiently developed to use them in the acute risk assessment scheme for aquatic invertebrates, fish and aquatic stages of amphibians to assess the risks of time-variable exposure. This presentation aims to illustrate the possible use of validated GUTS models, in combination with step 3 or step 4 FOCUSsw exposure profiles, as tools in the aquatic Tier-2 environmental risk assessment for an organophosphorus insecticide. This insecticide is characterised by short-term pulse exposures in edge-of-field surface waters. Experimental lower tiers indicate that aquatic arthropods (crustaceans and insects) in particular are at risk. The case study presented compares the outcome of the experimental effect assessment tiers (standard test species approach; geometric mean approach; species sensitivity distribution approach) with results of GUTS modelling to put these results into perspective. For the example insecticide selected, it appears that the application procedure of GUTS models in prospective ERA for pesticides as proposed by EFSA PPR (*EFSA Journal 2018;16(8):5377*) is promising to refine the risk of time-variable exposures. In addition, this Tier-2 assessment based on GUTS models was not in conflict with the principle of the tiered approach that lower tiers should be more conservative than higher-tiers. It is recommended that similar exercises are conducted with a representative number of substances differing in field exposure dynamics and toxic mode of action.

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A roadmap for the use of TKTD models in ERA of pesticides

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Tier-1 risk assessments for pesticide active substances and Plant Protection Products (PPPs) in surface waters rely on the quantification of treatment-related responses from standard protocol tests, where exposure is continuous. When a low risk cannot be demonstrated with this conservative Tier-1 data, one possibility is to address the mismatch between the constant exposure regime used in standard ecotoxicity tests and the time-variable concentrations predicted in the field. Toxicokinetic-toxicodynamic (TKTD) models can be used to help in refining the assessment by predicting individual-level effects under untested time-variable exposure conditions. In order take stock of the state of the art and to provide some guidance about the use of TKTD models in the risk assessment, the EFSA Panel on Plant Protection Products and their Residues (PPR) developed a dedicated Scientific Opinion (SO). This presentation aims at introducing the assessment strategy suggested in the opinion, focussing specifically on GUTS models. Ten different evaluation areas have been identified and organised in checklists for each TKTD model type. For GUTS, due to the standardised nature of such models, some of these have been considered addressed once for all in the remit of the SO. On the contrary, other areas need to be carefully considered in the context of any substance-specific assessment. Among those, particular attention is paid to the parameter estimation (model calibration), to the evaluation of the sensitivity and uncertainty, and to the evaluation of the model by comparison with independent measurements (model validation). Finally, some implications for the testing strategy are also discussed in relation to the application of TKTD models for risk assessment purposes.

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Measure or extrapolate? Extended parameterisation of TKTD models for population modeling under outdoor conditions

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Recently, the toxicokinetic-toxicodynamic (TKTD) model framework GUTS is recommended to be used in regulatory risk assessment by EFSA. In the last few years, individual-based population models (IBMs) are being increasingly applied in risk assessment that take into account physiological temperature dependencies as well as the size structure of populations. These models are often coupled to TKTD effect models, and can be used for extrapolating variable exposure scenarios to population effects under outdoor conditions. However, TKTD models usually take neither temperature nor size dependencies into consideration, although temperature and animal size can have a strong influence on the parameters of TKTD models. TKTD models normally coupled to individual-based population models describe only the sensitivity which has been measured for one specific organism size at only one constant temperature. For practical reasons, it is not always possible to test the smallest and most sensitive stages of organisms in the laboratory. In addition, for a realistic effect modeling with dynamic population models, it is necessary to know the sensitivity of all relevant body sizes. Measuring sufficient combinations of size and temperature is expensive and time consuming. An alternative could be the extrapolation of the required TKTD parameters from laboratory standard tests. Therefore, we examine concepts which seems to be suitable for extrapolation from the size analyzed to other life stages or size classes and between different temperatures. This presentation exemplarily examines the influence of seasonality on population-level toxicity under realistic field conditions, which considers the dynamic and variable timing of toxic exposure, body size composition in a population, and environmental stressors over the year. For this purpose, an individual-based population model for *Daphnia magna* is used, which has been coupled to a lake ecosystem model, including the TKTD model GUTS, which now has been extended by temperature and body size dependencies. This model approach was used to analyse different scenarios with i) measured, ii) extrapolated, and iii) ignored temperature and size dependencies of the TKTD parameters. Therefore, ecological as well as exposure scenarios for summer and winter conditions were selected, and population size and recovery time were considered as model endpoints to assess toxic effects on the population level.

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Toxicokinetic-toxicodynamic modelling of the effects of pesticides on growth of the laboratory rat, Rattus norvegicus

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Ecological risk assessment is carried out for chemicals such as pesticides before they are released into the environment. Such risk assessment currently relies on summary statistics gathered in standardised lab studies however, these statistics extract only very limited information and depend on duration of exposure so extrapolation to realistic ecological scenarios is inherently limited. Mechanistic effect models simulate the processes underlying toxicity and so have the potential to overcome such issues. Toxicokinetic-toxicodynamic (TK-TD) models operate at the individual level, predicting the internal concentration of a chemical over time and the stress it places on an organism. TK-TD models are particularly suited to addressing the difference in exposure patterns between lab (constant) and field (time varied) scenarios. So far we know very little about predicting sub-lethal effects of pesticide exposure to mammals, even though such effects are of particular interest with respect to longer term exposure. We demonstrate that TK-TD models based on the dynamic energy budget (DEB) theory can be used effectively with regulatory toxicity test data sets to predict toxic effects on the growth of rats over time. Model predictions can separate the impacts of feeding avoidance and toxic action, highlighting which was the primary driver of effects on growth. Such information is highly relevant to the ecological risk posed by a compound because in the environment alternative food sources may or may not be available to focal species. While this study focused on a single endpoint, growth, this approach could be expanded to include reproductive output. The framework developed is simple to use and could be of great utility for ecological research and to risk assessors in industry and regulatory agencies.

Latest Developments and Future Needs for Higher-Tier Studies, Risk Assessment and Risk Management in the Regulation of Biocides, Pesticides and Pharmaceuticals (I)

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Investigating the lethal and sub-lethal effects of anti-sea lice therapeutants, used widely on salmon farms, on the European lobster (Homarus gammarus)

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Sea lice are naturally occurring marine ectoparasites common in salmonid species, but in high numbers they present a serious problem for the salmonid farming industry. Sea lice infestations reduce the welfare of farmed fish and cause significant economic losses to the industry, estimated at €300 million annually around the globe. The industry heavily relies on the use of therapeutants added to the water or incorporated into fish feed to control sea lice infestations. These therapeutants enter the aquatic environment both directly, following the release of bath treatment effluents, or indirectly via the deposition of uneaten food pellets and/or faecal matter to the sea floor below the salmon pens. Although widely used, the acute and sub-lethal effects of these compounds on non-target crustaceans are not well known. We carried out a series of acute toxicity tests to assess the lethal and sub-lethal effects of deltamethrin and azamethiphos, two important neurotoxic pesticides, on European lobster (*Homarus gammarus)* larvae (stage I and II) following an environmentally relevant exposure scenario (1 h). Mortality and immobility were selected as lethal and sub-lethal toxic endpoints. We found that exposures to azamethiphos and deltamethrin were lethal to both larval stages examined, with 1h-LC_{50} values well below the concentration recommended to treat sea lice on salmon farms. Interestingly both compounds also caused significant immobilisation of larvae, and once immobilised the larvae were unlikely to recover up to 24 h post exposure. In field situations, immobile individuals would be incapable of maintaining their position in the water column, unable to avoid predators and unable to feed and therefore are considered to be ecologically dead. Since EC_{50} values (based on mortality + immobility) were substantially more sensitive than LC_{50} values, our results suggest that these compounds may pose a significant threat to non-target species and highlights the importance of considering EC_{50} values in risk assessment analyses of pesticides, particularly neurotoxic compounds such as azamethiphos and deltamethrin. We have also carried out an experiment examining the uptake and sub-lethal effects of emamectin benzoate (EMB), the leading in-feed anti-sea lice drug, in juvenile European lobster following a chronic (49 day) exposure. Sub-lethal effects examined included changes in gross morphology (body weight, carapace length) as well as transcriptional responses.

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Micro-dams on potato fields: consideration in environmental risk assessment as part of the MAgPIE toolbox
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On sloped agricultural fields, water and sediment can be transported downhill as run-off and erosion. This process can cause loss of valuable top soil material as well as transport of plant protection products (PPP) into adjacent surface water bodies. Farmers may use commercially available equipment to establish so called micro-dams between the rows of e.g. potato or maize fields to prevent run-off and enhance water infiltration. In European and US risk assessment for the registration of PPP, run-off is numerically calculated with the simulation model PRZM which uses the USDA run-off curve number (CN) concept for predicting the downhill transport of water and PPP with the runoff water movement. Results from run-off field trials were used to determine the effect of micro-dams between the ridges of potato fields on model input parameters. To this end, the relation between precipitation and runoff was used to derive CN. The impact on the actual risk assessment is shown with standard FOCUS-PRZM calculations of predicted environmental concentrations. A numerical study using the spatial model for runoff, floods and erosion openLISEM is shown to present an alternative way to estimate the run-off-reducing effect of micro-dams on potato fields. The results presented here support the approach to quantitatively consider in-field risk mitigation measures in the context of regulatory surface water exposure calculations, as proposed by the MAgPIE workshop. In the case of micro-dams, their mitigating effect can be quantitatively considered by reducing the CN in the exposure scenarios appropriately.

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Higher tier testing- When reality does not meet expectations: a tale of two copepods
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Higher tier testing is a valuable tool in assessing the risk of plant protection products (PPP) to aquatic and terrestrial organisms; however, higher tier testing comes with a unique set of challenges. One of these challenges is determining which species to use when developing a species sensitivity distribution (SSD) based on standardized laboratory tests. When selecting species, there are some aspects to consider, such as whether or not there is a published guideline or test method, if there are defined endpoints and acceptability criteria, and if the organisms are readily available from a reliable supplier. While these are all critical aspects of a higher tier test, the most critical question for the performing laboratory is "can we successfully conduct this test?" Smithers Viscient conducted a series of full life-cycle toxicity tests to produce an aquatic invertebrate SSD for a PPP. Among the species selected for this program were two copepods, *Tisbe battagliai* and *Amphiascus tenuiremis*. Guidance for copepod testing exists in the form of a new guidance document in the OECD Series on Testing and Assessment, Number 201. The methods described in this guidance document are specific to *Amphiascus* and have been developed and validated through a series of inter-laboratory tests. *Tisbe* was initially included as a possible species during development of the test method, but was ultimately excluded due to inconsistent performance and a lack of interest among validation participants. Published literature on the use of *Tisbe* for laboratory toxicity testing is available, but no formal test method exists; therefore, several modifications to the 201 guidance document were necessary to conduct testing with *Tisbe*. With a formal, validated guidance document supporting *Amphiascus* as an appropriate test species, an expectation for success was set. Given the lack of supporting information on test methods with *Tisbe*, there was a greater uncertainty of success with this species and its fit within the SSD. This presentation will review the results of the toxicity tests conducted with these two species.

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Optimising mesocosm study design to derive reliable and robust community endpoints
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Aquatic freshwater mesocosms are designed to represent edge of field water bodies by creating a habitat that will support communities of aquatic macrophytes, algae, zooplankton and macroinvertebrates in a contained system. The EFSA guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters [1], makes recommendations for a minimum of 8 sensitive/vulnerable taxa with acceptable minimum detectable difference (MDD) values to be tested. MDD values are useful in the interpretation of mesocosm data as they provide information on the probability that the statistical test will find differences between the treatment and controls. If MDD values are >100%, the power of the test is too low to demonstrate treatment related declines, therefore Brock provides further recommendations on the EFSA guidance for the improvement of MDD values and statistical power of mesocosm studies through improving the efficiency of sampling [2]. Sampling techniques that increase the number of individuals and reduce variability can reduce %MDD values. Therfore, it is vital to design and set up a mesocosm study that will support high species richness and abundance to achieve these requirements. Furthermore, the design of the individual mesocosm itself has an important role to play in the success of a study and the reliability of the data obtained. Here, we present case studies using historical CEA mesocosm data to highlight how improvemtents to mesocosm study designs based on the recommendations by Brock *et al*. affect MDD values. We will do this by conducting univariate statistics to calculate MDD values and comparing data from old mesocosm designs and new and updated mesocosm study designs. Improved mesocosm designs and sampling methodologies include using multiple plant health and growth measures, counting a higher proportion of phytoplankton sub samples and increasing the number of macroinvertebrate and emergent insect traps per mesocosm. Using these case studies we hope to demonstrate that our improvements to mesocosm study design will result in data that has the statistical power required to show community level effects. This is achieved by increasing the number of individuals counted in order to increase the statistical power and reduce %MDD values. In addition the improvement of measurement or sampling techniques that increase the number of endpoints can reduce variability and provide more reliable and robust data.

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The field study conundrum
F.M. Bakker, Bakker Consultant; S. Aldershof, Bioresearch & Evaluation
Arthropod field studies are unique in providing comprehensive ecological complexity at a relatively small spatial scale. Arthropod communities are not only highly speciose, they also cover a broad spectrum of activity patterns, habitat use and autecological traits that ensure full coverage of potential exposure scenarios. This diversity at the scale of the farm or below suggests a high degree of spatial variability that may be at odds with replicated plot study designs. Indeed, variability is an epithet applied to field studies and usually in negative connotation, however mostly without specification. We evaluate different parameters that may describe different aspects of variability, in particular abundance, species composition and response to exposure. First, we look at pre-treatment variability in species abundance and discuss strategies to cope with pre-treatment variability. In addition to variability in abundance, arthropod communities may also vary in species composition. Again, this is often regarded as a potential issue when it comes to extrapolating study results. We compare an array of arthropod communities at different sites over a period of 10 years and evaluate their compositional similarity at an appropriate taxonomic level. Subsequently, we compare community responses to reference insecticide treatment for a range of field studies and evaluate the variability in observed response. Although definitely more realistic than any lower tier study or model, a field study still represents a worst-case situation. The direct overspray of hay meadows to simulate spray drift events ensures uniform exposure over extended areas. Likewise, the high spray volumes and restrictions on maintenance treatments in studies in commercial cropping systems are worst case. In technical regard field studies can be considered sufficiently protective. However, the match of spatial scale with species mobility traits may constrain the practical scope of field study results. We investigate whether plot sizes chosen for typical study durations match with motility records and evaluate the temporal pattern of recovery from insecticide treatment for a suite of arthropod taxa. Finally, for certain species, effects at larger spatial scales can be expected on theoretical grounds. We evaluate the potential for overlooking such "action at a distance" by comparing mobility and sensitivity of different taxa relative to most sensitive species endpoints.

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Avian reproduction under real conditions: how to deal with variability and extrapolation in bird nest monitoring studies
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According to the relevant guidance in the EU (EFSA guidance document 2009, GD) nest monitoring is an option to verify acceptable risk of plant protection products (PPPs) to the reproductive performance of birds. Such a higher-tier study provides a maximum level of realism as all routes of exposure are included. Moreover, there are a range of well-established methodologies and statistical approaches for nest monitoring studies and sufficient sample sizes are relatively easy to obtain. However, as no detailed information is given in the GD about the appropriate study design there are concerns whether the natural variability can be covered and the extrapolation of results among different studies is questioned. Nest monitoring studies are thus an ideal example to discuss the regulatory expectations on higher tier studies and to demonstrate how guidance on the study design will help to deal with the issues of variability and extrapolations. A case study is used to illustrate the basic approach of nest monitoring studies in ERA for PPPs. Breeding parameters were monitored for tit species in conventional (applied with organophosphate [OP]) and organic pome fruit orchards in 2013 and 2014 in UK. The fate of nests was monitored at fixed intervals. The data evaluation included endpoints used in avian reproduction lab tests and advanced statistics for nest survival (logistic exposure models) for each species. The breeding parameters obtained for great tit pairs were compared with published literature for the same species in pome fruit orchards in France. In total, the fate of 465 active nests was monitored. Despite of the natural variability, the main pattern underlying the nest survival could be identified. The model of nest survival revealed a significant effect of the breeding date and year. The nest survival probability was not significantly affected by the OP application. The values of the measured breeding parameters in the pome fruit orchards in UK are very similar to those obtained in France. This similarity shows that a typical situation was studied and provides great confidence that the observed absence of effects can be extrapolated to pome fruit orchards in general in the central zone. These results and the comparison with published data shows that nest monitoring studies can be a valuable tool in ERA of the PPP risk to birds and therefore they should be part of the revision of the EFSA GD on birds and mammals.

Endocrine Disruption in Invertebrates: Historical Perspectives, New Developments, and Key Research Needs

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Levels and trends of tributyltin (TBT) and imposex in dogwhelk (Nucella lapillus) in Norway from 1991 to 2017
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The banning of organotin biocides, such as tributyltin (TBT), from use in marine antifouling paints is leading to reproductive health recovery in marine gastropod populations all over the world. TBT induces so-called imposex (superimposition of male sexual characters onto females) in certain marine gastropods, such as the common dogwhelk *Nucella lapillus*. Imposex is caused by the ecotoxic non-target effect of organotin antifoulants. In this study, the results of the Norwegian TBT and imposex monitoring in *N. lapillus* from the period 1991-2017 are presented. TBT-exposed females can develop irreversible and non-functioning male sex characteristics such as a vas deferens and a penis like organ. In late stages of development, females may become sterile or even die prematurely causing imposexed snail populations to decline and sometimes go extinct. This biological effect in *N. lapillus* is quantified by the Vas Deferens Sequence Index (VDSI) and Relative Penis Size Index (RPSI). The TBT/imposex monitoring was conducted at eight coastal stations representing the Norwegian coast. Subsequently, 50 specimens from each station was analysed individually for imposex/VDSI and pooled (only females) for TBT. In 2017, no sign of imposex (VDSI=0) was found in *N. lapillus* in any of the monitoring stations along the Norwegian coastline, for the first time since the monitoring started in 1991. There were significant downward trends for both TBT and VDSI, or low levels. Good correlations between TBT and VDSI, TBT and RPSI, and VDSI and RPSI in *N. lapillus* were found. The legislation banning use of TBT (in 1990, 2003 and 2008) have been effective in reducing imposex/VDSI in *N. lapillus* and have re-established some of the populations. This monitoring data confirm the rationale of implementing strict international regulations on industrial chemicals when these can be linked to ecological perturbations in coastal ecosystems.

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Do we need a paradigm shift in how we view mollusc endocrinology to protect molluscs from possible endocrine disruption?
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Molluscs make up a large and diverse phylum, second only to arthropods in the number of species. Molluscs are vital components of most ecosystems and are of great economic significance across the world both in terms of their value in aquaculture (e.g. oysters) and their impact as agricultural pest species (slug and snail damage) and as intermediate hosts for parasites (transmitting flukes and trematodes). Molluscs have also been subject to one of the best known cases of wildlife endocrine disruption caused by environmental chemicals, namely, the masculinisation of female marine gastropods, known as 'imposex', by the anti-fouling compounds such as tributyltin (TBT). Given the above, surprisingly there are still large fundamental knowledge-gaps in our understanding of mollusc developmental and reproductive endocrinology and misconceptions still prevail. For example, early gastropod imposex research initially suggested that TBT's masculinising effects were due to disruption to the steroid enzyme aromatase, and a subsequent increase in circulating androgen (e.g. testosterone) levels. In a vertebrate system this would certainly make sense as a driver of masculinisation. However, subsequent research has shown TBT's imposex effects are initiated via peroxisome proliferator–activated receptor (PPAR) and retinoid X receptor (RXR) interactions, and recent whole genome assessments for a number of mollusc species have found no homologous genes for aromatase and no mollusc nuclear androgen receptor. Nevertheless, ecotoxicology studies are still being published with mollusc 'aromatase' and testosterone levels given as biomarkers of chemical exposure. Recent research in our lab suggests that another steroid enzyme, 5-alpha-redutase which genomic evidence suggests molluscs do have, can be disrupted and has striking and highly reproducible developmental effects. I suggest that there is a pressing need to stop viewing mollusc endocrinology through a vertebrate lens and start focusing on the receptors, enzymes and transport proteins we know they have. We now have powerful molecular tools to investigate mollusc endocrinology, in order to understand the possible effect of environmental chemicals – we just need to start using them.

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Daphnia as a test organism for endocrine disruption
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Recently, there has been increasing evidence that chemicals that act as endocrine disruptors may have an effect in invertebrates, mainly insects and crustaceans. *Daphnia magna* has been proposed as a model organism for environmental ecotoxicology being a good biosensor for endocrine disruption because these chemicals may act by the juvenile and moulting hormone, endocrine hormones controlling development and growth. However, so far there is no direct evidence that all chemicals that are known endocrine disruptors in vertebrates truly act on this *D. magna* pathways. Here we address this question by first constructing gene expression signatures representing Daphnia response to both Juvenile and moulting hormones and then by comparing a panel of chemicals to the reference expression profiles of these two hormones. Interestingly, we identified Diazinon and Atrazine on one side and l-cyhalothrin and bifenthrin on the other one, to have a similar expression profile to either juvenile and the moulting hormone, respectively. We also set to experimentally validate our hypothesis. This finding is consistent with the working hypothesis that ED's act affecting these hormones signalling pathways. Our findings suggest that the approach we have developed could be used to identify novel endocrine disrupting compounds hence supporting the use of *Daphnia magna* for rapid screening in risk assessment

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Disruption of Biological Rhythms by Environmental Stressors: An Ecdysteroid-Regulated Biorhythm
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Biological rhythms are maintained by molecular clocks that regulate diverse biological processes that maximize individual survival and population sustainability. Disruption of these rhythms by environmental chemicals and other stressors can compromise population-relevant processes such as development, growth, and reproduction. The molecular constituents of some of these molecular clocks have been well described (e.g. circadian clocks); while, others remain poorly understood. Among the latter are infradian clocks, time-keepers that maintain biological rhythms of greater than 24 hours. We used the infradian rhythm of molting in the crustacean *Daphnia magna* to develop a model for the regulation of this rhythm and to identify possible targets of disruption of the rhythm by environmental stressors. Results generated to date, support a molecular model for the regulation of the molt infradian rhythm as follows. Each cycle within the molt rhythm is initiated by a pulse of the hormone 20-hydroxyecdysone. This hormone induces the expression of two known clock proteins E75 and HR3. E75 suppresses the transcriptional activity of HR3 through dimerization. This dimerization stalls the clock mechanism and allows for sufficient time to pass for the development of embryos prior to their release at the

end of the cycle. Nitric oxide binds to E75 causing the release of HR3 from the E75 causing the resumption of the clock. HR3 induces the expression of the transcription factor FTZ. FTZ then induces the expression of enzymes involved in the next 20-hydroxyecdysone pulse. This pulse demarcates the end of one cycle and the beginning of the next cycle within the rhythm. Exposure of daphnids to suppressors of E75 expression or function extended the time required for the sequence of molecular events, increased time to reproductive maturation, reduced growth, lengthening of the period between molts, and reduced offspring production. All effects were consistent with our model for the regulation of this infradian rhythm. Results indicate that E75 may function as a target of chemically-induced disruption of this, and possibly other biorhythms. The simultaneous disruption of the multiple processes regulated by the infradian rhythm by environmental chemicals could have profound adverse consequences on population sustainability

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Endocrine regulation and disruption of molting in arthropods: Mechanistic understanding and adverse outcome pathway development

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Molting is key to growth, development and reproduction in arthropods, and is precisely regulated by ecdysteroids through activation of the ecdysone receptor (EcR). Endocrine disrupting chemicals (EDCs) mimicking the actions of ecdysteroids have therefore been developed as pesticides to combat harmul arthropods. These chemicals, however, may also affect nontarget organisms due to highly conserved endocrine systems in arthropods. Due to the high number of potential EDCs and arthropod species in the environment, it is not possible to perform hazard assessment for all chemical-species combinations. Better mechanistic understanding of molting disruption by different EDCs and in different taxa, and the development of universal models for arthropods in general are needed. The present study therefore employed the adverse outcome pathway (AOP) concept with the main aims of investigating the molting regulatory pathways in arthropods and evaluating a conceptual AOP for molting disruption using the aquatic crustacean *Daphnia magna*. An extensive literature survey was performed to summarize existing knowledge and develop a conceptual pathway network (CPN) for understanding the molting regulatory processes in arthropods. The conservation of key proteins involved in the regulation of molting was assessed using the SeqAPASS tool. A conceptual AOP was developed based on the CPN and a number of key toxicity pathways leading to lethal molting disruption were highlighted. The AOP was submitted to the AOPWiki (AOP #4). Experimental evaluation using *D. magna* showed that four known insect EcR agonists were able to bind to the *D. magna* EcR. The *in vivo* study revealed an apparent increase and subsequent decrease of the ecdysteroid titer required for a succesful molt cycle in *D. magna*, which complied with knowledge from insects and other crustaceans. Exposure of *D. magna* to the endogenous ecdysteroid 20E showed impaired molting, ovulation, feeding, cuticle structure, and increased mortality. Multiomics analysis (RNAseq, iTRAQ proteomics and lipidomics) further revealed that a number of genes, proteins and lipid metabolites were mapped to the CPN, indicating similar endocrine regulation of molting in *D. magna* compared to other arthropods. The present study has provided substantial mechanistic insights into molting regulation and disruption in arthropods and developed the first invertebrate endocrine disruption AOP to facilitate next generation hazard and risk assessment of EDCs.

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Using endocrine disrupting parasites to inform endocrine disrupting chemicals: a molecular to population level approach

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Many aquatic invertebrates are prone to infections by parasites which can alter sex ratios and behavior along endocrine-mediated pathways. These parasites, despite sometimes ignored or considered an inconvenient ‘noise’ in environmental monitoring have the capacity to inform and conceptualize the impacts of endocrine disrupting chemicals. For example, incomplete feminization by parasites can sometimes leave populations with a very high prevalence of intersexuality. Intersex specimens carry of a burden of altered growth, reproduction, and immunity which can be modelled. In addition, behavior-altering parasites utilizing serotonin-mediated pathways can leave organisms with a 20-fold increased likelihood of predation. The molecular, individual and population-level impacts of these parasites have been well characterized within the fields of ecological research. Here we provide a body of research into crustaceans which have feminizing and behavioral altering parasites that are being used to determine adverse outcomes (AOs) for reproductive and neurological endocrine disruption using transcriptomic and population modeling approaches. Using the amphipod (*Echinogammarus marinus*) as a model, we have determined the differential gene expression of specimens with parasite and non-parasite mediated intersexuality. We highlight that existing vertebrate biomarkers of feminization should not be applied to crustaceans, as orthologous genes are not induced in feminized amphipods. Furthermore, in contrast to vertebrates, where feminization and intersexuality are often associated with deleterious de-masculinization, we find males maintain masculinity even when unambiguously feminized. This reveals a considerable regulatory separation of the gene pathways responsible for male and female characteristics and demonstrates that evidence of feminization (even if detected with appropriate biomarkers) is not a proxy for de-masculinization in crustaceans. Moreover, we have used behavioral and serotonin altering parasites to develop behavioral tests to inform serotonin altering pollutants such as antidepressants. These intern have revealed insightful biological pathways using high-throughput sequencing approaches. All these reproductive and behavioral costs have been used to parametrize a dynamic deterministic population model over 10-year scenarios striking effects on long-term populations.

Measuring the Sustainability of Circular Economies: the Potential of LCA

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Improving the effect of circular procurement: Three aspects of measuring sustainability impact

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‘He who pays the piper calls the tune”. The Dutch government spends 73 billion Euro per year, while at the EU level the amount totals to 1.8 trillion Euro per year. This large public spending power is strategically supportive to stimulate sustainable procurement, forwarding policy goals such as increased resource efficiency through a circular economy and climate change reduction. Sustainable procurement is already practiced for more than 15 years in the Netherlands and other EU countries a-like. And circular procurement, which is one form of sustainable procurement, attention. For circular procurement sustainability impact measurement has three applications during the procurement procedure. These are 1) potential impact assessment in order to determine for which products or services circular procurement (which is often an investment in terms of capacity) is most beneficial; 2) comparative impact assessment, to choose between optional products and services; 3) to evaluate the actual impact of the sustainable and circular procurement during and after contract management and stir for further improvement. Life Cycle Analysis (LCA) plays a role in all three aspects, but different with regards to scope, requirements for the inventory, and quality assessment. In 2018 the actual effect of this policy instrument, sustainable procurement, among which circular procurement, was evaluated in the Netherlands for 16 product groups. 150 procurements where analysed, of which 117 showed to take sustainability into account. The contracting authorities of these procurements were contacted to check if the finally supplied products or services where indeed more sustainable than the market average. LCA was used to estimate the difference between the supplied and the market average. This difference is an indication of the effect of sustainable procurement, which was e.g. expressed in CO_2 equivalents 5 megaton over the whole contracts of the products and services supplied. Hence, public procurement has great potential to contribute to sustainable transitions, like toward a more circular economy. By measuring the actual impact, lessons can be learned for realising this potential.

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Interpreting LCA results from a life cycle and circular economy perspective: Application of the Life Cycle Gap-Analysis

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The interpretation of LCA results from a LCG-A perspective allows to analyze the consequences of product innovations & new technologies with regard to both, the vision of circular economies and the actual consequences for current life cycle systems. In other words: the LCG-A visualizes the distance between an ideal closed system and the status quo in a simplified way, without ignoring trade-offs across the entire life cycle. LCG-A thereby supports various stakeholders from research and development, industry and politics by analyzing the consequences of their ideas and decisions for individual products. The application of the LCG-A is demonstrated based on a virtual case study of a lithium-ion (li-ion) battery for e-mobility.

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Using relative market prices to better evaluate the environmental benefits of the circular economy

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To verify whether recycling or other Circular Economy strategies are environmentally beneficial, we need to compare the life cycle impacts of the CE activity with a scenario in which this activity is not implemented. Functional comparability is a key issue in any comparative LCA – especially comparing a recycled (or reused, remanufactured, etc.) product with a primary alternative – but also in the modelling of substitution related to the use or the supply of a recycled material. A general assumption in LCA is that a recycled product is 1:1 comparable to its primary equivalent. However, this assumption is often inaccurate due to differences in quality or even image. Furthermore, changes in material properties can cause that the recycled material is competing with a primary material of a completely different nature. We propose to use the relative market price between the recycled product and the substituted primary product (A) as an indicator for functional comparability. If the products have the same price (i.e. $A = 1$), the products are fully comparable, and substitution could be modeled according to the end-of-life recycling method. A different price (i.e. $A \neq 1$) could indicate that the recycled product substitutes a product in a different market segment due to a changed functionality, quality, or a different image. In this case, a closer substitute could be identified in a different market segment. Alternatively, the use of the recycled product could lead to downstream drawbacks and/or benefits, which should be integrated both into the calculation of the market-price ratio and into the life cycle inventory. Finally, if the user of the recycled product experiences a financial benefit (i.e. $A < 1$), the demand for the recycled product is (partly) constrained, which is especially relevant in a consequential LCA. The latter implies that there is a surplus of the material and substitution is (partially) modelled by avoided or additional waste treatment. This approach enables to make two product systems more comparable by considering realistic substitutes and downstream effects of using a recycled product. The market-price ratio A can be easily integrated into the "Circular Footprint Formula" of the Product Environmental Footprint Guide of the European Commission to better model substitution in an LCA. Finally, this approach provides the perspective to put a price on externalities, which would improve the representation of factor A.

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Quantifying net environmental and economic benefits of food waste prevention actions
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A methodology to evaluate the effectiveness of food waste prevention actions based on Life Cycle Assessment (LCA) and cost benefit analysis is presented. This enables to quantify the net environmental benefits of an action by considering three components: (i) the environmental impact embedded in the avoided food waste up to the stage of the supply chain where the food waste is diverted, (ii) the environmental impact associated with the waste treatment of the avoided food waste and (iii) the environmental burden of the action implementation. Similarly, a cost benefit analysis is performed considering: (i) the cost of the food items saved, (ii) the cost of the avoided waste treatment and (iii) the cost of the action implementation. Following this approach, a tool was developed to enable users (mainly non-LCA experts) to perform the evaluation of a specific food waste prevention action by introducing the following information: the amounts and types of food items diverted from waste, the stage of the supply chain at which the food waste is avoided, the country where the action takes place, the type of waste treatment that would have been used had the waste been generated, the resources used in implementing the action and the overall cost of the action. An example of application of the methodology developed and the related tool is provided, by quantifying the environmental and economic benefits of an existing food waste prevention action. The influence of some of the action parameters over the results are tested through a sensitivity analysis, to identify the most relevant aspects to consider when designing an action in order to maximise its impact in terms of environmental and/or economic gains. The adoption of a life cycle approach minimizes the risk of burden shifting (i.e. by identifying situations in which the prevention action presents a burden on the environment that outweighs the benefits achieved) and can support in the design of effective food waste prevention actions. Finally, the results provided by the tool can be used to communicate the positive impact achieved through an action.

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Life Cycle Assessment of asphalt pavements containing plastic waste as secondary raw material
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In Europe, in the last decade, an increasingly share and quantity of post-consumer plastic waste has been sent to recycling, the 31.1% of all plastic waste collected. Nevertheless, most of what is collected is still sent to landfill or energy recovery and even a part of plastic waste supposed to be recycled is incinerated. Therefore, to avoid the loss of potential secondary raw materials, research should explore new sustainable recycling paths, according to the circular economy approach. One of this path can be the utilisation in the asphalt mixture of plastic waste otherwise destined to incineration, in order to improve the performance and thus lifespan of asphalt pavements. The present study uses the LCA methodology to assess a new process, developed to produce an asphalt that contains a super-modifier made out of recycled plastic, which improves the asphalt performance. The main goal is to quantify the environmental impacts of the new and the conventional processes and to compare them, focusing on the Italian context. The evaluation encompasses the asphalt mixture production process, from raw material extraction/collection to the production of asphalt mixture, and, besides the conventional flows (aggregates, bitumen and reclaimed asphalt pavement), it includes the additional flow of plastic waste used to produce the super-modifier. The functional unit was defined as one kilometre of extra-urban road; impacts are also expressed in terms of performance, referring to ESALs (Equivalent Single Axle Load). Preliminary results of the ongoing analysis shows that, for all the impacts categories of both CML2001 and Impacts ILCD/PEF recommendation impact assessment methods, the environmental impacts of the asphalt containing the super-modifier is lower than the conventional one, considering also the estimated lifespan related to the performance improvement. At this stage, the assessment indicates that the use of plastic waste as an ingredient in the asphalt mixture is able to enhance the structural performance while reducing the environmental impacts. The inclusion of the phases following the production process, namely road construction maintenance and EoL, will represent the next step of the analysis and allow a more accurate and complete assessment.

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Poster spotlight

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Target and NonTarget Mass Spectrometry for Human and Environmental Exposure Assessment (I)

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Complementary methods for extending chemical space coverage using EPA's Non-Target Analysis Collaborative Trial mixtures
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The EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) was designed, in part, to characterize current methodological strengths and weaknesses and enables participants to identify knowledge gaps in their analysis workflow. Composed of 1269 unique ToxCast chemicals, ENTACT samples comprise 10 mixtures (numbered 499 to 508) of varying complexity in terms analyte number and presence of isomers and/or isobars. These mixtures were analysed using hydrophilic interaction chromatography (HILIC) and reversed phase (RP) chromatography with a quadrupole time-of-flight mass spectrometer to investigate how chromatography affects analyte detection. In mixture 505, with 365 total analytes, 13 of 14 highly polar compounds (negative log K_{OW}) were not observed in RP (+ESI) but showed a marked increase in ionization efficiency (300 to 2000% improvement in ion count), and improved retention in HILIC. On the contrary, analytes like 1,1,3-trimethyl-2-thiourea and acetazolamide (log K_{OW}= -0.09 and -0.37, respectively) were observed in RP alone. Further analysis of the mixtures was performed with atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) connected to an Orbitrap mass spectrometer; 136 compounds ionized exclusively in APCI while 192 ionized exclusively in ESI. In total 540 unique compounds were observed in both ionization modes. Cheminformatic fingerprinting was performed to determine if functional group trends may help predict whether a molecule will ionize in APCI or ESI. For example, molecules containing the following substructure groups: ring: hetero_[6]_N_triazine_generic, ring: hetero_[6]_N_triazine_(1_3_5-), bond: CN_amine_sec-NH_aromatic_aliphatic, bond: CN_amine_aromatic_generic preferentially ionize in APCI. These results can be used to predict the ionization behaviour for untested chemicals to improve the chances of successful detection and can also provide further evidence to identify unknown chemicals. In addition, adjustments to the spectral processing software RMassBank during the ENTACT trial further revealed that organometallic species should be considered in non-target studies using LC-HRMS. Taking both chromatographic and ionization approaches together, it is demonstrated that chemical space coverage is improved using complementary approaches. We conclude that HILIC should be used to complement RP instead of as an alternative. In the same manner, APCI should also be used in conjunction with ESI to expand coverage.

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Why we can't cover industrial chemicals in mass spectrometry screening, and what to do about it

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A traditional non-target screening of an environmental sample (e.g. waste water) normally ends up in a surprisingly few industrial chemicals. The Swedish Chemicals Agency (KemI) have therefore started up a collaboration with analytical reseach laboratories to found out the reasons and what to do about it. One observtion made is that current reference libraries for identifcation are not god enough for identification of industrial chemicals. One reason for this may be that chemical identity and structure data are mainly stored in regulatory databases with limited public access. To test this hypothesis KemI created a suspect list chemicals in commerce according to our different regulatory databases, called KemI Market list. The list contains more than 30.000 susbtances. The original list contains CAS number, chemical name and monoisotopic mass. Two prioritisation score where added to support the prioritisation among all candidates. One on the probability of a correct match, based on use pattern (*Exposure Score*), and one on hazard based on the Hazard classification system CLP (*Hazard Score*). The database matching (against adduct corrected m/z data) was made at KemI. KemI made a primary prioritisation on the probability and the regulatory concern, and return the result to the laboratory for futher identification. The results show that the number of identified industrial chemicals increase. An other limitation observed is that industrial chemicals in regulatory databases normally are identified with CAS numbers. CAS numbers are not always related to a specific structure. This is especially common for industrial chemicals. This make it difficult for a authority to use CAS numbers to found the souce in the society for an identified chemical. The over all conclusion is that non-target screening for regulatory purposes demands better communication and further standardisation.

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Quality assurance and quality control of non-target screening of emerging contaminants in human urine by LC-HRMS

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The concept of contaminants of emerging concern (CECs) refers to compounds that are not (yet) included in monitoring programs because they are new or their presence in the environment has not been elucidated or understood. However, they may have the potential to exhibit toxicity in humans and wildlife. The impacts of human exposure to mixtures of chemicals are poorly understood, because biomonitoring campaigns do not include CECs. Therefore, there is an urgent need to establish a set of representative biomarkers to assess the human exposure to mixtures of CECs. Human urine is a complex matrix and the expected concentration for most of the CECs is at trace levels. In addition, presumably many types of contaminants are metabolized through different pathways and (partially) excreted through the urine. Non-target screening analysis of human urine samples by high resolution mass-spectrometry is able to provide an overview of the presence of CECs in the population. However, despite the attractive features of this novel strategy, it is facing a lack of harmonized methods that would permit obtaining comparable and high quality results. Quality assurance(QA) is defined as a set of activities or procedures which are adopted in a laboratory to ensure that all quality requirements will be fulfilled. Meanwhile, quality control (QC) refers to operational techniques and activities that are used to fulfill requirements for quality. To facilitate the development of reliable and comparable non-target/suspect screening workflows for the assessment of CECs in human urine by liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS), we will further develop a generic QA/QC framework. The achievement an actual and representative fingerprint of CECs in human urine is a challenge that requires the establishment of the proper QA/QC for each individual step of the workflow. Although the establishment of a detailed list of QA/QC represents a good starting point to get to a harmonized approach of non-target/suspect screening methodologies in human urine analysis. However, more effort in this direction is still needed owing to the premature status of the major workflows in this field.

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Improving Confidence for Phthalate Metabolite Identification by Fragmenention Pathways in LC-HRMS Based Non-Targeted Screening of Human Urines

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Humans are exposed to thousands of synthetic chemicals such as plasticizers that are used in many commercial products. Targeted monitoring methods using gas/liquid chromatography – mass spectrometry are sensitive ways to provide quantitative information in surveillance of well-known chemicals in exposure science, but only a small portion of chemicals in commerce have ever been monitored by this technique. Top on hundreds of new chemicals created from industries every day, the transformation of legacy compounds under environmental conditions can also produce thousands of unknown new compounds. With growing interest in causes of human diseases from exposure to contaminants in the environment and the needs of many governments to evaluate exposure risks to emerging substances and new chemicals to ensure human health, non-targeted screening methods using high-resolution mass spectrometers have become important in suspect-screening and identification of unknown metabolites in human biofluids as exposure biomarkers of emerging contaminants for risk assessment. While workflows have been developed for these methods, high-throughput database match for suspects and structure elucidation for real unknowns in complex matrices are still challenging. Especially, the lack of first-order MSMS fragment spectra from reference compounds impedes unambiguous assignment of unknown metabolites in human samples. The fragmentation pathways in MS/MS scan from the same group chemicals with similar structural skeletons are considered to be promising tools to fill this gap with the objective of increasing the identification confidence for unknown metabolites of certain group of chemicals. In this presentation, new workflows have been established to improve the identification of unknown compounds derived from LC-HRMS-based non-targeted screening analysis in both full-scan and auto-MSMS scan modes. Different fragmentation pathways and their fragmentation patterns are compared on identification confidence of unknown metabolites. The specific fragment ions from the same structural skeleton in the same group have been applied to increase the confidence of unknown metabolite identification. The performance of the structural identification workflow has been tested and validated in groups of phthalate metabolites in human urine samples. The fragmentation pathway strategy greatly decreased the amount of false positives in screening process.

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Hundreds of Unrecognized Halogenated Contaminants Discovered in Polar Bear Serum

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Exposure of polar bears (*Ursus maritimus*) to persistent organic pollutants was discovered in the 1970s, but recent evidence suggests the presence of unknown toxic chemicals in their blood. Protein and phospholipid depleted serum was stirred with polyethersulfone capillaries to extract a broad range of analytes, and nontarget mass spectrometry with “fragmentation flagging” was used for detection. Hundreds of analytes were discovered belonging to 13 classes, including novel polychlorinated biphenyl (PCB) metabolites and many fluorinated or chlorinated substances not previously detected. All analytes were detected in the oldest (mid-1980s) archived polar bear serum from Hudson Bay and Beaufort Sea, and all fluorinated classes showed increasing trends. A mouse experiment confirmed the novel PCB metabolites, suggesting that these could be widespread in mammals. Historical exposure and toxic risk has been underestimated, and these halogenated contaminants pose uncertain risks to this threatened species.

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Identification of bioaccumulative organic contaminants along the aquatic foodweb using high-resolution mass spectrometry

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Aquatic organisms are exposed to mixtures of thousands of chemicals. Among them, some legacy contaminants (PCBs, etc.) are known to bioaccumulate and biomagnify along the trophic chain raising risk for wildlife and human. However, the actual exposure of aquatic organisms to more emerging contaminants remains scarce while exposure studies mainly focused on surface water compartment that not reflect the actual internal dose. Therefore, it appears relevant to get a more comprehensive picture of the actual mixtures to what aquatic organisms are exposed. In this context, this study aims to implement and evaluate an High Resolution Mass Spectrometry (HRMS)-based strategy to identify chemicals that bioaccumulate and biomagnify in aquatic organisms. To this end, sediment, biofilm, leave, macroinvertebrates and fish samples extracted by QuEChERS were analysed by using high performance liquid chromatography coupled with electrospray to a QExactive HRMS detector (ThermoScientific). HRMS analysis consisted of data dependent acquisition (DIA) in positive mode allowing to get merged MS2 scans. First, these data were evaluated under Trace-Finder 4.1® through the comparison of our experimental data to a home made database based on the accurate mass, retention time, isotopic pattern, and MS2 scans when available. From the initial list of 359 chemicals with exact mass hits, data dependent acquisition (DDA) acquired MS2 spectra were obtained for 145 candidates allowing to confirm 56 of them that were quantified by using internal calibration. Among them, biocides, fungicides and herbicides were the more widely detected along the trophic chain. Then, DIA data were also processed for suspect/non-target screening under Compound discover 2.1® From the 503 915 identified features, filtering (area, background, predicted composition and mass list match) allowed to reduce the list to 1200 features on the basis of a Norman mass list (17500 chemicals). Hierarchical clustering analysis further allowed to identify a cluster of 20 candidates with increasing area along the trophic chain and to define an inclusion list of candidates for additional MS2 acquisition that is ongoing. Our results provide a proof of concept for the implementation of HRMS-based strategies to fill the gap of knowledge about exposure of organisms to emerging contaminants. Such strategy could be the way to identify relevant contaminants for investigating their fate into organism and along the trophic chain.

Ecosystem Services: Progress, Case Studies and Reflections

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European Assessment and management of risks to ecosystem services: Reflections
S.E. Apitz, SEA Environmental Decisions Ltd; L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences
The benefits which flow from natural capital, termed ecosystem services (ES), are under threat from habitat loss, climate change, invasive species, over-exploitation and pollution. At the same time they are increasingly the focus of environmental policy. Among the top 10 research questions identified by the SETAC Europe Horizon Scanning Project was “Biodiversity and ecosystem services: What are we trying to protect, where, when, why, and how?” An ES approach has been used to frame specific protection goals for ecological risk assessment (ERA) and the inclusion of ES into the life cycle impact assessment (LCA) framework is a current activity in the LCA community. ES are also being used to frame broader sustainability, cost-benefit and stakeholder engagement. It is therefore an opportune time to bring the ERA and holistic assessment communities together. An objective of the EU 7th Environment Action Programme is ‘to protect, conserve and enhance Europe's natural capital’ and the 2020 Biodiversity Strategy has a target of ‘halting the loss of biodiversity and the degradation of ecosystem services in the EU by 2020’. Do we, however, have sufficient knowledge and understanding to assess, predict and manage the effects of anthropogenic activities on ES delivery in multifunctional landscapes that are often exposed to multiple pressures, and are the monitoring, regulatory and decision frameworks that have developed over decades fit for purpose as we seek to apply this evolving knowledge? Assessing and managing the risks that stressors, often introduced as people seek to obtain selected services from land- and waterscapes, pose to the sustainable delivery of these and other ES is a transdisciplinary challenge requiring new approaches to communication and collaboration. A recent SETAC Europe special session explored how close we are to adopting an ES approach to assessing and managing environmental risk from anthropogenic stressors. It drew on the findings of a series of SETAC-Europe sponsored workshops (**C**hemicals: **A**ssessment of **R**isks to **E**cosystem **S**ervices) and built on the recent work by EFSA and ECETOC in using ES approaches to develop specific protection goals and the outcomes of the joint ESA-SETAC Pellston workshop Ecosystem Services, Environmental Stressors and Decision Making. Thus, it reviewed the state of the practice for the use of ES (primarily) in Europe, for prospective risk assessment, chemicals regulation, retrospective risk assessment (also termed forensic ecology), remediation and restoration. This paper will summarize key points, focusing on progress, opportunities, gaps and challenges, with a focus on case studies, underlying assumptions, and the relationship with ES, sustainability and LCA thinking.

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Building on the LCA experience to initiate a standardization process for ecosystem services
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The demand for rigorous ecosystem services (ES) assessment approaches is tremendously growing. This mainly comes from a broad community of ES practitioners other than researchers, among which public and private sector investors, multilateral and intergovernmental authorities, regulators, landowners and land users, which require best-in-class, customized and harmonized standard monitoring and management practices, instruments and guidelines to systematically incorporate ES considerations in their decision-making processes. Channelling ES knowledge into policy and decision-making practice further requires the harmonization of definitions, the standardization of classification processes and the streamlining of methodological and epistemological properties of ES accounting, quantification, valuation and mapping approaches. Recent efforts to combine the ES concept and assessment methodologies with the life cycle assessment (LCA) method show that examples and avenues to overcome the current taxonomic and methodological gaps exist. LCA is a widespread methodology standardized by ISO 14040:2006, which is hugely applied around the world by thousands of industrial actors and decision makers across numerous economic sectors and products. LCA allows converting a variety of environmental stressors into a set of specific integrated indicators of environmental impact. This facilitates impact monitoring and communication to stakeholders. It also enables the comparison of environmental performance, the development of labels and certification schemes, as well as implementation of environmental protection policies, regulations and technological improvements. In this presentation, we discuss options and challenges to advance the application of ES science and the potential for the ES community to learn from the LCA experience in ISO-level standardization in order to promote methodological harmonization. Our objective is to initiate a dialogue and cross-fertilization exercise between the two research communities that could open up opportunities for collaboration in the development of ES management standards. <br clear="all" />

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Soil Quality and related Ecosystem Services in Life Cycle Assessment
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Soils are unquestionably an essential component of the global ecosystem and high quality functional soil is important for the supply of ecosystem services such as, providing biomass and regulating climate. Therefore, maintaining healthy and functioning soils is a fundamental requirement for globally sustainable production chains, particularly those related to agricultural and forestry based systems. The related land use and land use change (LULUC) are key human stressors that can affect the quality of soil and hence it’s functioning and delivery of ecosystem services. A task force working within Life Cycle Initiative hosted by United Nations (UN) environment has been working for the last two years to recommend suitable indicators which can be used within a Life Cycle Assessment framework (LCA) which can be used for soil quality and its associated ecosystem services. The dynamics of soil organic carbon (SOC) accumulation and loss are a function of soil type, climate, land-use type, and in particular land management (occupation and change), which makes it a good proxy for damages to ecosystem services. Therefore, as changes in soil organic carbon (ΔSOC) stock is interim, the task force recommends its use as an indicator of soil quality as it is a good integrative indicator of soil functions. Further suggestions by the task force are that soil loss is recommended as a separate indicator linked to natural resources, to address erosion impacts. Additional issues identified by the task force have been the need for improving the representation of forestry and permanent crops.

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Discussion

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Biogeochemistry and Fate of Organic Pollutants in Aquatic Systems (I)

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Assessing the contribution of biotransformation to the fate of 40 micropollutants in a lake ecosystem
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Organic micropollutants (MPs) such as pesticides and pharmaceuticals are consistently measured at the ng/L to μg/L level in freshwaters. Studies on lakes such as Greifensee in Switzerland have attempted to measure the contribution of different dissipation pathways to the mass balance of several pharmaceuticals over consecutives months. However, the contribution of biotransformation to the overall dissipation of MPs has rarely been quantitatively elucidated. This project aimed to fill this knowledge gap by conducting biotransformation experiments during several, ecologically differing phases of plankton succession in Greifensee, and at different depths. Grab samples of Greifensee water were collected during 6 sampling campaigns extending from April 2018 to October 2018. Concentrations of 40 MPs were measured at 5 different depths of Greifensee, and the original water from 3 m and 6 m (filtered at 80 μm to remove zooplankton) was spiked with the mix of 40 selected MP, each at an initial concentration of 100 ng/L, and used for biotransformation experiments as batch cultures in an environmental chamber. MP concentrations were measured at 8 different time points over the course of 3 weeks using LC-MS. No biotransformation was observed for caffeine and other conservative compounds. Moreover, compounds known to be rather efficiently phototransformed, e.g., diclofenac, were found to behave conservatively under the low light conditions used. In contrast, for compounds such as valsartan, biotransformation was observed during some experiments and biotransformation rate constants could be calculated. The kinetics obtained from the batch experiments helped to understand the observed concentration changes measured in Greifensee. For example, MPs such as valsartan and sulfamethoxazole showed strongly deviating seasonal concentration profiles, even at depths clearly extending into the hypolimnion. Their concentrations were lowest when the biomass of microorganisms was particularly high in Greifensee (i.e., late summer), suggesting that these compounds might undergo

biotransformation by the native microorganisms. By combining biotransformation rate constants measured in laboratory batch cultures with lake mass balance considerations, we were able to confirm the importance of biotransformation relative to phototransformation for the fate of several MPs in a lake ecosystem.

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Spatial and seasonal distribution of organic micropollutants in Lake Mälaren, Sweden

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The main objective of this study was to investigate the occurrence and fate of organic micropollutants (OMPs) in surface water in Lake Mälaren over one year and assess their seasonal variations and spatial (vertical and horizontal) distribution. The water samples were enriched with solid phase extraction (SPE) and subsequently analyzed by ultra-high pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The applied multi-residue method comprised 122 organic micropollutants (OMPs) covering a wide range of physico-chemical properties that were chosen due to their high annual usage rates and continuing major concerns regarding their adverse effects on humans and aquatic organisms. In total, 50 OMPs were detected at least once above limit of quantification (LOQ). The highest detected concentration was found for lamotrigine with 140 ng/L. The locations Ekoln and Västeråsfjärden were identified to be most affected by OMPs pollution. Seasonal patterns were observed for numerous OMPs indicating varying pollution sources and compound specific distribution and degradation processes of OMPs in the aquatic environment. Only a few vertical distribution patterns and concentration gradients were observed, for instance, the deepest sampling depth (30 m) from Ekoln showed considerable higher concentrations than the upper sampling depths in February. A strong positive correlation was found for carbamazepine and lamotrigine, but also for other OMPs indicating similar pollution sources or transport and fate processes. Two industrial chemicals, tolyltriazole and tris(2-butoxylethyl)phosphate showed very good analytical performance parameters and were detected frequently and it is recommended to incorporate these compounds more regularly in future analysis. To the best of our knowledge, this study is the first one to report the occurrence and distribution of OMPs representing such wide physico-chemical properties, including industrial chemicals, in a Swedish lake.

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Analysing biotransformation kinetics of micropollutants across different water-sediment test systems

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To date, it remains impossible to fully prevent bioactive and therefore potentially harmful substances (i.e. pharmaceuticals and pesticides) from entering the aquatic environment. Some pathways through which those pollutants reach surface waters are hardly controllable such as agricultural runoff. The manufacturing and use of chemicals, however, could be controlled more easily. From the regulatory side, the OECD 308/ 309 guidelines represent the most relevant simulation studies to assess persistence of chemicals that may enter surface waters. There is consensus that transformation kinetics observed in those OECD studies do not directly project into compound behavior in the environment. A thorough understanding of relevant system-specific differences that govern the transferability from laboratory to field systems is still missing. This work targets the connection between persistence measured in different regulatory water-sediment test systems and for different types of sediment by investigating a persistence evaluation concept that employs a substance-specific bioavailability-corrected and biomass-normalized second-order biotransformation rate constant (k'_{bio}). Various OECD 308 and 309-type experiments were set up and the fate of 38 substances, mostly pharmaceuticals and pesticides, was followed. The measured concentration-time series and other parameters such as bacterial abundance within the test systems were used to feed an inverse modelling framework estimating transformation rate constants. The results of the model fitting suggest that k'_{bio} may be a generally valid parameter for quantifying biotransformation across different water-sediment systems and a valid indicator of a substance's aerobic biotransformation potential. The system-independency of k'_{bio} makes it a promising future tool to translate transformation kinetics from the laboratory to the field.

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Long-term mercury records in a boreal lake: lake processing and the role of organic matter

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Fish in northern humic lakes often have mercury (Hg) levels higher than advisories for human consumption. The Hg in these lakes is primarily from current and legacy long-range transported air pollution sources. Foodweb exposure to aquatic Hg is regulated by dissolved organic matter (DOM) as a transportation vector, source of energy, and regulator of bioavailability. Long-term records of Hg in inlets, lakes, and outlet enable understanding of the role of catchments and lakes in regulation of foodweb exposure to Hg. The inlet and outlet streams of lake Langtjern in southern Norway have been monitored for water chemistry and hydrology since the 1970s and since 2004, for MeHg and total Hg (monthly since May 2008). Langtjern is oligotrophic, recovering from acid deposition and browning. The ratio of mean annual lateral catchment Hg inputs and direct atmospheric deposition of Hg to the lake is circa 8:1. Long-term mean concentrations of HgT and MeHg in the outlet are 3.5 and 0.08 ng/L, respectively, and show no changes over time. However, the ratio of MeHg to HgT shows a significant linear decrease ($p< 0.05$). Annual patterns in inlet and outlet concentrations are similar, suggesting that lake processing does not have a major impact on outlet Hg dynamics, but is rather driven by catchment processes. However, the Hg:dissolved organic carbon (DOC) ratio in the outlet is significantly higher than in the inlets, an indication of lake DOC removal through in-lake processing, and redistribution of Hg to remaining DOC. Size fractionation of DOM (tangential ultrafiltration) and studies of biodegradability of size fractions (measured as O_2 consumption) indicate differences between inlet and outlet in size fractionation, biodegradability of fractionated DOM and Hg associated with the different size fractions. The smallest size fraction (2 consumption per g DOC). We suggest that the base of the aquatic foodweb in the outlet is more exposed to Hg than in the inlets, especially for organisms relying on bacteria as energy source. Bacterial consumption of easily degradable, Hg-contaminated DOM is thought to be an important pathway for Hg into the aquatic foodweb.

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Investigating the micropollutant biotransformation potential of differently adapted natural stream biofilms

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Micropollutants, found in complex mixtures in the environment, can pose a risk to aquatic organisms. Stream biofilms, which consist of algae, bacteria and other microorganisms, play a key role in nutrient cycling and have been demonstrated to have a large potential to act as natural sinks for micropollutants. Exposure of stream biofilms to treated wastewater has been shown to result in increased tolerance to micropollutants, possibly due to an increased biotransformation potential of those biofilms. In our research, we want to explore the ability of differently adapted natural river biofilms to biotransform micropollutants. Thus, in spring 2017, we launched a study involving stream biofilms cultivated up- and downstream of two wastewater treatment plant outfalls, which was replicated in fall 2018. The main aim of this study was to investigate the biotransformation potential of natural river biofilms towards a large mixture of environmentally relevant micropollutants. Our experiments consisted of suspended biofilms in batch reactors with adequate controls. A mixture of 55 compounds was spiked into the reactors and concentrations were monitored over time. In general, we found a broad range of micropollutants to be biotransformed. Fourty compounds showed a concentration decrease, that, given the data from the controls, could be attributed to biotransformation. Of these, six were not consistently biotransformed between all sites. Within the 34 compounds that showed decreasing concentration patterns in all of the investigated biofilms, a variety of primary biotransformation reactions were represented, such as conjugation, substitution-type, and oxidation-type reactions. Interestingly, three of the six compounds that showed different biotransformation patterns between the investigated sites belonged to the compound class of artificial sweeteners. In particular, for these compounds, biotransformation could only be found in the downstream communities. Those findings lead to the conclusion that the respective downstream communities underwent changes that lead to an increased potential to biotransform those compounds, whereas the upstream communities did not. Thus, our studies were able to point out the relevance of stream biofilms in biotransforming micropollutants and lead to some interesting open questions regarding the origin of the increased biotransformation potential towards specific micropollutants in communities exposed to treated wastewater.

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Fate of atmospheric aromatic hydrocarbons into the oligotrophic ocean

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Polycyclic aromatic hydrocarbons (PAHs), and other semivolatile aromatic-like compounds (SALCs), are an important and ubiquitous fraction of organic matter in the environment which fate in the ocean remains uncharacterized. Here, we report a global assessment of the occurrence and atmosphere–ocean fluxes of 64 PAHs analyzed in paired atmospheric and seawater samples from the Atlantic, Pacific and Indian Oceans. The global atmospheric input of PAHs to the ocean is estimated at 0.09 Tg/month, four times greater than the Deepwater Horizon spill. The environmental concentrations of total SALCs were 10^2–10^3 times higher, with a relevant contribution of an aromatic unresolved complex mixture. These concentrations drive a global deposition of carbon estimated at 400 Tg C yr^{-1}, around 15% of the oceanic carbon uptake due to CO_2. Furthermore, we identify the biological pump and microbial degradation as key sinks of aromatic hydrocarbons in the oceans. Plankton and seawater samples showed lower concentrations of the hydrophobic compounds in the seawater when plankton biomass was higher, consistent with the relevance of the biological pump. However, the mass balance for the global oceans showed that settling fluxes of aromatic hydrocarbons in the water were 2 orders of magnitude lower than atmospheric deposition. These observations show the relevance of PAHs degradation processes in the oligotrophic oceans. Among different processes considered (i.e. photochemical degradation) microbial degradation appeared to be the key factor for the depletion of the bioavailable hydrocarbons. We quantified the relative abundance of PAHs-degrading genes by analyzing the frequency of the alpha and beta subunits of RHD (specific biomarkers of PAH degradation) in the public metagenomic database of the oceanographic expedition Tara Oceans. Degradation genes for PAHs were found to be ubiquitous. The several lines of evidence provided support a relevant microbial degradation of PAHs, and also relevant for the largest SALCs organic carbon pool. It has been reported that large oligotrophic oceanic regions are heterotrophic, requiring sources of allochthonous organic matter. According to this work, degradation of atmospheric inputs of aromatic compounds is quantitatively relevant for the marine carbon cycle, and could help explain the observed heterotrophy of the oligotrophic oceans.

Remediation of Contaminated Environments and Innovative Methods in Biological Strategies (I)

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Cropping trees and fiber plants at phytomanaged sites

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Phytomanagement-based restoration programs for contaminated soils may use woody plant species that are colonized by essential microorganisms, especially in highly stressed and nutrient-poor environments, such as TE- contaminated sites. Trees from phytomanaged sites have been proposed as a potential source of biomass for bioenergy and various energy-recovery techniques such as combustion, gasification and pyrolysis, which we have recently addressed. Within previous projects, we exploit the implementation of an integrated bioremediation option that combines poplar together with mycorrhizal fungi. We implemented 3 demonstration sites in France of 1 ha each mostly contaminated by Hg and As. Four replicate field blocks were established in February (P) and March (F) 2011 using unrooted poplar cuttings Skado (*P. trichocarpa x P. maximowiczii* section Tacahamaca) and I214 (*P. deltoides x P. nigra* sectionAigeiros). Most interestingly, 5 years after the plantation, biotic and abiotic conditions have promoted the appearance of a spontaneous herbaceous cover. In the present work, we show recent data on an on-going project (http://phytofiber.fr/), which main objective is the multi-purpose valorisation of wood and plant fibres produced on mixed-metal-contaminated soils. Despite the unfavorable soil conditions, we found that poplars performed quite well at the phytomanaged sites in terms of biomass production, that was enhanced by inoculation with symbiotic fungi. The naturally occurring nettle under poplar offered a natural source of fibers, which properties are equal to or better than those of hemp and flax, making this nettle biomass very promising for composite application.

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Operating on bioavailability processes during bioremediation: Roles of pollutant phase exchange and microbial dispersal

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This overview presentation will cover innovative strategies for a controlled enhancement of pollutant bioavailability, with the aim at reducing the execution periods (and costs) of bioremediation for the achievement of acceptable limits for risk reduction, and even reach lower end-points than currently possible. For bioavailability to be incorporated into bioremediation, three questions must be addressed: (1) how is "bioavailability" defined? (2) how should it be measured? and (3) is it possible to increase bioavailability but not environmental risk of the pollutants? In a recent review, the concepts and definitions of bioavailability of organic chemicals are examined from a risk assessment perspective (*Environ. Sci. Technol.* 49:10255-10264, 2015). The main schools of thought consider bioavailability (focusing on the aqueous or dissolved contaminant), bioaccessibility (incorporating the time for exposure), and chemical activity (determining the potential of the dissolved contaminant for biological effects). These concepts are the basis for different methodologies (desorption extraction, passive sampling and biological tests) and mechanistic studies on the different bioavailability processes (contaminant soil/sediment interactions, transport and passage across cell membrane, and biological responses such as toxic effects or biodegradation). Our group has proposed different ways to operate at different levels on these processes for a better bioremediation performance in risk reduction. The approach is relevant because in some circumstances bioremediation may even increase risk of the pollutants. The prospected risk-minimizing strategies include targeting slow-desorption pollutant fractions with (bio)surfactants (*Environ. Sci. Technol.* 45:3019-3026, 2011; *Environ. Sci. Technol.* 48:10869-10877, 2014), the localized fertilization of free-oil phases or NAPLs (*Environ. Sci. Technol.* 45:1074-1081, 2011), modulated deposition and motility of microbial degraders (*Environ. Sci. Technol.* 46:6790-6797, 2012; *Environ. Sci. Technol.* 52: 10673−10679, 2018), promotion of bioavailability with plants and root exudates (*Soil Biol. Biochem.* 57:830-840, 2013; *Environ. Sci. Technol.* 49:4498–4505, 2015), and facilitated microbial (bacterial/oomycete) interactions for the colonization of pollutant interfaces (*Environ. Sci. Technol.* 50: 7633-7640, 2016; *Environ. Sci. Technol.* 51: 11935-11942, 2017).

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Role of rhizoremediation in PCB contaminated areas: remediation time estimation from greenhouse experiment data

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In the last two decades there has been a growing interest in bioremediation technologies which use plants and microorganisms to degrade organic chemicals such as PCB in contaminated sites (rhizoremediation). Different studies have been conducted to investigate the potential of plant-microbe interactions in the remediation of organic chemical contaminated soils with respect to natural attenuation, providing useful data such degradation half-lives. Such a type of data can be used to predict soil concentration temporal trend and the time needed to achieve legal limit when using plants and their associated rhizosphere microbe to remediate contaminated sites. In the present work a greenhouse experiment was performed with an aged contaminated soil coming from a National Relevance Site (SIN) for remediation located in Northern Italy (SIN-Brescia Caffaro). The soil was characterized by high concentrations of PCB at mg/kg levels. 10 different treatments (combination of different plant species and different soil conditions) were considered together with the appropriate controls (soil without plants). Soil and plant biomass were analysed at the beginning of the experiment (t0) and 18 months after the seeding (t18). The obtained soil concentrations were used to calculate concentration reduction with time and the corresponding rhizoremediation half-lives. The rhizoremediation half-lives obtained for the most efficient treatments were used to estimate the time needed to achieve legal limit considering three different areas characterized by a high spatial variability of PCB concentrations. Geostatistical techniques were used to build up remediation time maps for the three different areas. The aims were to: 1) provide a rhizoremediation half-live dataset for PCBs, 2) evaluate the effectiveness of rhizoremediation when a complex contamination gradient is present, 3) compare the influence of natural attenuation vs. plant/microbe interactions on remediation time. This study provided an important rhizoremediation half-life dataset for PCBs referred to different plant species and soil conditions. Moreover, it showed that when a complex contamination gradient (spatial variability of concentrations, contaminant mixture, etc.) is present, rhizoremediation must be accurately implemented and fine-tuned (in terms of species to be selected, their density, etc) to account for effective remediation.

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Characterization of a rhizosphere microbial community of a poplar-assisted bioremediation strategy applied for recovering a contaminated soil from PCBs

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A poplar-assisted bioremediation strategy has been applying in a historically PCB contaminated soil located in Southern Italy. At different times, PCB concentrations were evaluated in soil samples at different depths and distance from the tree trunks of selected contaminated plots. Similarly, microbial analyses were carried out on soil samples to assess total microbial abundance, cell viability, dehydrogenase activity and the phylogenetic composition of the autochthonous microbial community. An overall improvement in soil quality in terms of a significant decrease in all PCBs investigated and increases in microbial abundance, cell viability and activity was observed. The highest microbial abundance and total nitrogen content were found in the rhizosphere. In all soil samples, the PCB values are under the Italian legislation limits in each plot considered. RNA and DNA were extracted and cDNA synthesized. The hypervariable regions V4-V5 of the 16S rRNA gene were amplified using universal primers U519F and U926R. The microbial community structure was characterized by MiSeq (Illumina). Using DNA or cDNA different information was available. *Proteobacteria* was the most abundant phylum in both DNA and cDNA samples, *Acidobacteria* and *Bacteroidetes* were more present in the rhizosphere than in the bulk soil. *Firmicutes* and *Actinobacteria* were more present in bulk than in rhizosphere soil. Significant differences were found between rhizosphere and bulk soil at different hierarchical levels. In particular some genus such as *Mesorhizobium*, *Skermanella*, *Pseudomonas*, *Stenothtrophomonas* able to degrade PCBs were found in the rhizosphere, showing the role of these bacterial populations in the contaminant removal.

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Utilizing low-cost natural waste for the removal of pharmaceuticals from water: Mechanisms, isotherms and kinetics at low concentrations
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The presence of pharmaceuticals in the aquatic environment has been well documented and is of global concern due to their adverse impacts including antibiotic resistance. Conventional wastewater treatment processes are often not sufficient to eliminate these contaminants, and hence, the need to develop effective solutions remains. To address this challenge, sorption processes may possess certain advantages in terms of effectiveness, convenience and simplicity, however, their application is sometimes limited by high cost (e.g., activated carbon costs 700-5000 US$/ton). Alternatively, other biosorbents may offer a sustainable solution. This study evaluates the potential use of low-cost materials derived from agro- and/or industrial wastes as low-cost biosorbents for the removal of priority pharmaceuticals of legislative concern. Unlike many other studies, we do not focus on the synthesis of high cost functionalized sorbents, instead we seek to demonstrate how "waste" (without additional processing) can be used to remove pharmaceuticals at environmentally relevant, low concentrations. The performance of eleven natural low-cost biosorbents to remove 17 priority pharmaceuticals from aqueous media was investigated and the biosorption behaviour of the three most efficient materials was considered using two model compounds (diclofenac and trimethoprim): biochar (pyrolyzed from orchard prune residues), macro-algae (brown seaweed, *Fucus vesiculosus*) and wood chippings (oak tree waste). This work considers the biosorption behaviour for these quite different pharmaceuticals, considering (in depth) the mass transfer principles, kinetics, isotherms and the mechanisms involved. High removal efficiencies were achieved at low initial pharmaceutical loadings, indicating the potential for application of these biosorbents at low (i.e., environmentally relevant) pharmaceutical concentrations. These materials may thus provide a sustainable (circular economy based), low cost water treatment solution, especially in rural or semi-rural areas.

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Use of adsorbents for accelerated bioremediation of petroleum-contaminated soils
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In spite of the evident privilege of bioremediation for petroleum-contaminated soils, application of this approach is restricted due to poor adaptation of indigenous or inoculated microorganisms and plants in the highly contaminated soils. The main goal of this presentation is to outline up-to-date level of knowledge on the use of adsorbents for bioremediation of petroleum-contaminated soils as well as to share our experience of investigations in that direction. Several long-year laboratory and out-door experiments were carried out with various types of mineral soils spiked with crude oil, diesel fuel, and spent motor oil at the initial concentrations of petroleum hydrocarbons (TPH) from 5 to 15_{mass}%. We studied the Influence of soil amendment with biopreparations (BP) based on hydrocarbon degrading microorganisms as well as with natural adsorbents of various origination (mineral, organic, and carbonized) were studied. The experimental results indicate that the maintenance of optimal hydrothermal conditions, aeration, soil pH, ratio C:N:P:K are the most important factors for successful process of bioremediation. The soil amendment with BP appeared effective only in some cases: moderately high petroleum content (from 7 to 10% TPH) or in cold climate. Additional acceleration of bioremediation can be created through soil amendment with natural adsorbents of all classes. Their optimal doses deviated from 0.2 to 9% depending on the adsorbent and form or rate of contamination. Positive influence of the adsorbents is explained: 1) by the reduction of soil toxicity to microorganisms and plants due to mostly reversible adsorption of toxic petroleum components and especially their oxidized products; 2) - reduction of soil hydrophobicity followed by improving soil porosity and water capacity; 3) – localizing water soluble components in the treated soil layer. Hereby no significant accumulation of toxic polyaromatic hydrocarbons has been detected in soil and grasses grown after soil recultivation. The research was supported by RFBR grant No16-05-00617a

From Hazard Assessment to Regulatory Risk Management Action - Approach Development for UVCBs (I)

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What makes it challenging to regulate UVCBs ? – A perspective from regulatory authorities on how to regulate in an effective and proportionate manner multi-constituent and UVCBs
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Environmental hazard assessment (classification, risk assessment (PNEC), PBT assessment) together with information on uses and potential for releases and exposure is the starting point for authorities to initiate regulatory risk management actions. Any decision to start a regulatory action implies some level of certainty on the hazard or risk identified. It is also important to have a clear knowledge on the composition of the substance to be regulated so that industry is able to fulfil its obligations and authorities to enforce the regulatory measures. In comparison to mono-constituent substances, the environmental hazard and risk assessment of substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), can be more challenging, because (1) the number of constituents may be large, (2) the composition (partially) unknown and/or (3) variable (due to e.g., seasonal and temporal variation in sourcing of raw materials). Those specificities make it very challenging, if not impossible, to address the hazard properties and risks of those substances. Consequently, for UVCBs a different test strategy and a more weight of evidence approach is followed to assess the hazard. Indeed, testing the whole substance does often not provide information on the properties of individual constituents. Furthermore, the composition of test solution might be different from the composition of the substance added or from the environmentally relevant composition. On the other hand, when constituents are structurally similar, it may also not be necessary and efficient to test all constituents separately. However, it is of upmost importance that uncertainties associated with various approaches used for the assessment of such substances, including the weight of evidence approach, are understood by regulators to ensure that appropriate regulatory actions can be taken. Discussions are ongoing between authorities and other stakeholders to find strategies / approaches to overcome the challenges to determine the hazards and risks of UVCBs and to reflect on how to best use the data generated to serve more than one regulatory processes (Harmonised classification and labelling, PBT assessment, risk assessment). For instance generating toxicity data that would be of use for PNEC derivation, classification and the T assessment under PBT assessment. Whether this is possible is still under discussion and will be reflected together with the use of specific testing methods such as testing the water accommodated fraction (WAF).

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Difficulties in regulating UVCBs: Industry perspective
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Chemical risk assessment is based on the hazard and exposure of the substance. The approaches for these assessments is derived predominantly from methods for single substances such as crop protection chemicals, that have the potential for long range transport, bioaccumulation and biomagnification. However, significant challenges are encountered when these same approaches are applied to substances that do not fit this profile, such as with substances of unknown or variable composition, complex reaction products and biological materials (UVCBs).

Challenges arise in the test design, the interpretation of hazard data, the determination of the fate of the material and the quantification of exposure. Although the Organization for Economic Cooperation and Development (OECD) offers guidance on how to adapt existing test methods for difficult substances, there is limited guidance on how to conduct risk assessments for UVCBs. Industry experience has shown that the nature of UVCBs defies a single approach for risk assessment, necessitating tailored hazard and fate assessments with variable regulatory acceptance. One approach is to assess the persistence (P), bioaccumulation potential (B) and toxicity (T), referred to as PBT assessment, of UVCB constituents. This approach relies on Weight of Evidence (WoE) to provide both a clear understanding of the composition of the substance and to identify when constituents meet PBT criteria. WoE is especially critical given that existing methodology for hazard assessment of UVCBs is poorly defined. Additionally, care should be taken regarding using precautionary principles to identify PBTs and consequently designate UVCB substances as a Substance of Very High Concern (SVHC) because these assessments do not account for exposure. Since >20% of the substances on existing chemical inventories are UVCBs, there is a significant need to improve the current approaches for hazard and risk assessment of UVCBs. This presentation gives a brief overview of UVCB hazard assessment approaches that are fit-for-purpose and pragmatic. These include adapting existing methodology for P, B, and T testing, grouping substances into categories, and *in silico* approaches. The issues encountered with each of these approaches will be discussed, along with an overview of proposals for improvements being developed based on recent scientific research supported by industry.

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Petroleum substances: ongoing program to address hazard assessment including how to capture variability in composition

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CONCAWE addresses variability in hazard assessment of UVBCs in part through development of a comprehensive library of representative constituents. These structures are expected to capture the variability of different potential isomers across substances. QSARs are used to estimate P, B, and T properties for the constituents within a block. Where available, empirical data are used to support this assessment in a tiered approach. Persistence is evaluated using primary half life data from freshwater and seawater, which are then extrapolated to soil and sediment. This database include more than 1200 individual entries across more than 10 major hydrocarbon classes. Bioaccumulation is evaluated using fish BCF data (from aquatic and dietary exposures) and BMF/TMF data in weight of evidence to evaluate secondary poisoning concerns. Blocks that are potentially indicated as P and B are then evaluated for T using empirical data and QSAR. This evaluation is updated on a regular basis as new science and data are developed. Future work plans will also be discussed.

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The Environmental Assessment of Natural Complex Substances under REACH

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Natural Complex Substances (NCS) of botanical origin are a very diverse family of substances that are notably used as ingredients in fragrance formulations, which are incorporated into personal care and household consumer products. Because of their biological nature, several factors such as the region of growth, the climate within the region, the part of the plant used as source material and the process applied lead to natural variations in their chemical composition. Their complexity varies widely from simple substances with only a few constituents to very complex substances comprising more than 100 constituents which cannot be fully characterised. Further, constituents of an NCS may exhibit different physico-chemical, fate and effect properties that are relevant for their environmental assessment. The European Federation of Essential Oils (EFEO) and the International Fragrance Association (IFRA) have published guidance to help companies, many of whom are small and medium sized enterprises, fulfil their legal requirements for the environmental assessment of natural complex substances under REACH (https://echa.europa.eu/-/guidance-on-environmental-assessment-for-essential-oils-now-available). The guidance, prepared by environmental experts from the fragrance and essential oil industries in close cooperation with the European Chemical Agency (ECHA) was specifically aimed to support the 2018 REACH deadline, under which the vast majority of these substances fell (tonnage band below 10t). The ECHA Substance Identification Guidance generally considers that NCS fit the sub-category of "UVCB sub-type 3". However, they may also be considered as mono- or multi-constituent substances based on their composition and level of characterisation. The nature of the NCS will affect the approach chosen for environmental assessment. Factors such as knowledge of constituents, difference in properties amongst them, technical limitations in testing and feasibility to generate new data will influence the strategy for any given NCS. The guidance document considers assessment based on specific constituents, on the basis of blocks of constituents and on the basis of information on the NCS itself. It provides illustrations and examples of how they might be applied for the purposes of environmental classification and labelling, PBT assessment and environmental risk assessment. Specific considerations and issues related to NCS are also discussed. In this presentation case-studies taken from the guidance will be used to demonstrate the challenges in performing environmental assessments of natural complex substances.

Micro(Nano)plastic Pollution: Tackling the Plastic Problem by Identifying Sources, Investigating Fate and Novel Approaches (II)

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Degradation and toxicity of microplastic fibres and their role as a source of emerging pollutants

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Microplastic fibres (MPFs) are increasingly being reported as one of the dominant forms of microplastic pollution in aquatic environments. Clothing and textiles produced from synthetic fibres such as polyester (PES), polyacrylic (PAC) and nylon (PA) are considered some of the main sources of MPFs. However, there has been little focus on the environmental fate and effects of MPFs compared to microplastic particles and fragments. In the current study, the environmental fate of MPFs was studied by long-term UV degradation studies using a suite of 5 MPFs with different physical and chemical characteristics (PES, PAC and PA) under freshwater and marine conditions. A detailed chemical characterisation of the pristine test materials was conducted, using GC-MS, LC-MS and ICP-MS to identify the type of additive chemicals present. Degradation studies were conducted in sterile synthetic freshwater (algal culture medium, TG201) and sterile-filtered seawater. Exposures were conducted in 35 mL quartz glass tubes using an Atlas Suntest CPS+ with Xenon lamp generating 65 W/m^2 light intensity and fitted with a daylight filter (300-400 nm). The endpoints studied after 5 and 10 months were changes in MPF size (light microscopy and SEM), polymer chemistry and surface properties (ATR-FTIR), and the leaching and degradation of additives (colorants, UV-stabilizers, softeners). The release of additive chemicals and their possible photodegradation to intermediate products was investigated as part of the UV degradation studies. In addition, we investigate the potential for the same suite of MPFs and their leachates to elicit toxicological responses in a marine microalga. Effects on algal production, photosynthetic activity and lipid content were assessed as a function of polymer type, fibre length, concentration, as well as the additive chemical profiles of the different MPF leachates.

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Transgenerational effects of secondary microplastics and natural particles on Daphnia magna

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Most standardized ecotoxicological tests, such as the "*Daphnia magna* reproduction test" (OECD Guideline 211), cover up to one generational cycle, only. Additionally, they are not adapted to analyze the impacts of suspended particles. A number of publications so far have shown a decrease of feeding activity in Daphnia in the presence of suspended materials which may lead to adverse effects. While standard toxicity testing optimizes among other parameters food supply to ensure health and high reproductive output, these conditions are not representative of the natural environment. This is especially true if the agent under investigation is a suspended solid that is suspected to at least partially act via reducing the energy uptake. Thus, artificially high food availability during exposure plus initially high maternal energy reserves would buffer effects modulated through energy uptake during a short time frame. Accordingly, we exposed four consecutive generations of *D. magna* to three concentrations (400, 2000 and 10000 particles mL^{-1}) each of irregular polystyrene microplastics and kaolin as natural reference particle. The individuals in the treatment groups were fed a reduced amount of algae (0.05 mg carbon $individual^{-1}$ day^{-1}) while we also included a regularly fed control group (0.2 mg carbon $individual^{-1}$ day^{-1}; according to OECD 211). In the exposure to the highest microplastic concentration, we observed an extinction of the first generation daphnids within 21 d. The reproduction, growth and survival of daphnids exposed to 2000 particles mL^{-1} declined over two generations and completely collapsed in the third generation. Interestingly, exposure to a similar concentration of kaolin did not affect *D. magna* over the course of the experiment. As expected, reduced food supply resulted in a decline in reproductive output after the first generation in all treatments. Kaolin treatments showed reproduction that was higher than both the low-food control and the PS treatments. Overall, the PS particles combined with food limitation negatively affect exposed populations to the point of extinction while kaolin did not have such effect.

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Potential impact of micro- and nanoplastic particles on metal speciation and bioavailability in aquatic media

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Plastic particles collected from aquatic systems are typically found to carry a range of associated metal species, some of which are potentially toxic to biota, e.g. Cd^{2+} and Pb^{2+}. The metal content of microplastics (MPs) and nanoplastics (NPs) arises from a combination of the inherent components of the original polymer formulation (stabilizers, pigments, etc.) as well as the sorption of metal species from the environment onto and into the polymer matrix. Metal species may also be associated with organic matter that is sorbed on the surface of the particles. It follows that the amount of metals associated with MPs and NPs will evolve as a function of their residence time in the water column, with consequences for the chemical speciation, bioavailability and potential toxicity of trace metals in aquatic ecosystems. As a first step to gaining mechanistic insights into the involved processes, we present an elementary model, based on spherical geometry, to describe the association and release kinetics of metal species from MPs and NPs. The focus is on release kinetics of metal ions from MPs and NPs that have been immersed/suspended in an aquatic system for an extended period, in the context of their ingestion by biota. Within the body of the plastic particle, metals may be present as e.g. free (partially hydrated) ions and inner-sphere complexes with the polymer backbone and/or various additives. The overall release kinetics of metal species from the plastic particles is governed by the relative magnitude of the rate of diffusion within the particle body as compared to the rate of dissociation of inner-sphere complexes. The effective diffusion coefficients for metal species within plastic polymers are many orders of magnitude lower than those applicable for hydrated metal ions in bulk aqueous media. Thus, for not too strong inner-sphere complexes, the rate of diffusion of metal ions within the particle body will largely govern the overall release kinetics. We analyse practical examples in this context. The outcomes highlight the significance of particle size and residence time within biota for predictions of the potential bioavailability of metal species associated with MPs and NPs.

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How ingestion kinetics influence microplastic bioaccumulation and toxicity in Antarctic krill (Euphausia superba)

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The discarding of plastic products has led to the ubiquitous occurrence of microplastic particles in the marine environment. The uptake and depuration kinetics of ingested microplastics for many marine species still remain unknown despite the importance for understanding bioaccumulation potential to higher trophic level consumers. In this study, Antarctic krill (*Euphausia superba*) were exposed to polyethylene microplastics in order to quantify acute toxicity and ingestion kinetics, providing insight into the bioaccumulation potential of microplastics at the first-order consumer level. In the 10 day acute toxicity assay, no mortality or dose dependant weight loss occurred in exposed krill, at any of the exposure concentrations (0, 10, 20, 40, or 80 % plastic diet). Krill exposed to a 20% plastic diet for 24 hours displayed fast uptake (22 ng mg^{-1} h^{-1}) and depuration (0.22 h^{-1})rates, but plastic uptake did not reach steady state. Efficient elimination also resulted in no bioaccumulation over an extended 25 day assay, with most individuals completely eliminating their microplastic burden in less than 5 days post exposure. Our results support recent findings of limited acute toxicity of ingested microplastics at this consumer level, and suggest sublethal chronic endpoints should be the focus of further ecotoxicological investigation.

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Translocation of 14C-nanoplastics in the scallop Pecten maximus at environmentally realistic concentrations

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Studies that investigated the uptake and effects of nanoplastics in marine organisms have been conducted at what may be unrealistically high concentrations. This is due to the analytical challenge that represents tracking plastic particles in biological matrices at environmentally relevant concentrations. Here, we present the results of pulse exposures of ^{14}C-radiolabeled nanopolystyrene to the bivalve *Pecten maximus*, at what have been predicted to be environmentally relevant concentrations (< 15 μg L^{-1}). Our results show that the uptake was rapid and greater for 20 nm than for 250 nm particles. Autoradiography of bivalves sampled after the 6 h exposures showed accumulation of 250 nm nanoplastics in the intestine, while 20 nm particles were dispersed throughout the whole-body, possibly indicating translocation across epithelial membranes. Yet, depuration was also relatively fast for both sizes; 20 nm particles were no longer detectable after 14 d, although some 250 nm particles were still detectable after 48 d. As such, the particle size apparently influences the biokinetics and recommends further work on chronic exposures. Modelling extrapolations suggested that it could take up to 300 d of continued environmental exposure to reach a steady state although the concentrations would still be below 2.7 mg g^{-1}. Comparisons with previous works in which bivalves were exposed to non-plastic (silver) nanomaterials of similar size (20 nm), suggest that nanoparticle composition may also influence its biokinetics.

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Poster spotlight

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Effect Modelling for Regulatory Risk Assessment: Current Applications and Future Directions (II)

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Simplifying DEBkiss to model sublethal effects of chemicals

N. Sherborne, Syngenta / Environmental Safety; N. Galic, Syngenta Crop Protection Inc. / Environmental Safety

The recent scientific opinion published by EFSA has cleared the General Unified Threshold model of Survival (GUTS) for use in ecological risk assessment (ERA)) for aquatic invertebrates, fish, and aquatic stages of amphibians. However, in chronic toxicity studies the most sensitive endpoint is often a sublethal effect on an organism's size or its fecundity. We present a modelling approach based on DEBkiss and inspired by the procedure used by GUTS models. The simplicity of the model means that non-experts should be comfortable using and understanding the the model and interpretting its results. Using simplified DEB theory means that models can be calibrated based on standard laboratory study data alone. We demonstrate the suitability of the model using a chronic toxicity study of a hypothetical herbicide as a case study. This simplified and self-contained method could form the basis of a user friendly tool for sublethal effect modelling and prediction, following the path of projects to produce user friendly GUTS tools.

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The combined potential of species-traits and mechanistic effect models

S. van den Berg, Namur University; A. Focks, Wageningen Environmental Research / Environmental Risk Assessment Team; P. van den Brink, Wageningen Environmental Research

A key challenge in ecological risk assessment (ERA) is to assess the potential risk of chemicals to the wide range of species in the environment on the basis of laboratory toxicity data derived from a limited number of species. Recently, it has been proposed that the inclusion of species traits in ERA could provide a useful description of species assemblages in nature and has the potential to replace classical taxonomic approaches. Additionally, there is a growing recognition that the use of mechanistic approaches in ERA, especially quantitative models, may improve predictive and extrapolative power. An example of such quantitative mechanistic effect models are toxicokinetic (TK)- toxicodynamic (TD) models of the General unified Threshold models of Survival (GUTS) framework, which link external exposure and survival effects by describing dynamically the process of TK (uptake, biotransformation, and elimination) and TD (damage/hazard, internal recovery and thresholds). We hypothesise an amplified combined potential of GUTS models and trait-based approaches, due to the mechanistic match between species traits and GUTS parameters. We test this by performing linear regressions between i) classical sensitivity endpoints (LC50 and EC50) and species traits, and between ii) GUTS parameters and species traits. Additionally, we implemented a cross-validation step that uses predicted GUTS parameters as an input for the prediction of sensitivity endpoints. The whole analysis is done for twelve freshwater arthropods exposed to chlorpyrifos. We find that neither of the standard sensitivity values, i.e. the LC50 or EC50, showed a strong correlation with traits (option i), whilst multiple quantitative links between traits and/or trait combinations and process based GUTS model parameters could be established (option ii). Our results demonstrate the combined potential of mechanistic effect models and trait-based approaches. However, results from the cross-validation and the final prediction of sensitivity endpoints from GUTS parameters show that methods still can be improved further, e.g. by the further division into functional groups or extension of trait collections. Additionally, the model should be validated more extensively by adding other species.

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Recovery assessment in stochastic population modelling
M. Kattwinkel, S. Kunz, University of Koblenz-Landau / Quantitative Landscape Ecology; O. Jakoby, Rifcon GmbH / Regulatory Sciences - Population Modelling and Statistics
Environmental risk assessment of pesticides allows some population level effects to non-target species under certain conditions (recovery option; EFSA 2016) when it can be shown that the population under investigation recovers within an acceptable period of time. Population modelling is a valuable tool in addition to field and semi-field studies to assess recovery. However, so far there is no explicit guideline available how to estimate recovery times from simulated population dynamics, and the methods applied in the scientific literature differ greatly. In particular for stochastic population models (e.g. individual-based models, IBMs) with virtually unlimited numbers of replicates there might always be a statistically significant difference between sets of control and treatment populations. For a sound and reproducible evaluation of simulation model results with regard to population recovery it is important to understand the consequences of several choices: (1) the accepted difference between control and treatment to consider populations as recovered; (2) the method used to estimate this difference; (3) the consideration of stochastic processes resulting in short-term divergence. In this study, we investigate how robust and conservative different methods for the estimation of population recovery times are using an IBM that simulates population dynamics after the application of a hypothetical pesticide. We introduce different methods based on hypothesis testing, percentiles and quotients, and discuss their specific characteristics when assessing recovery. Additionally, we investigate the role of thresholds (maximum allowed difference between control and treatment) and other control parameters (e.g. percentiles used to aggregate over replicates and minimum time period to ensure persistent recovery). We also discuss the impact of the models variability as well as seasonal fluctuations in the population dynamics. Finally, we give recommendations on what methods lead to ecologically meaningful and robust results.

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Evaluation of a population model for ecotoxicological risk assessment
E. McVey, J. Wassenberg, Ctgb; A. Barsi, Ctgb / Dept of Theoretical Biology; D. Kulkarni, Ctgb; A. Focks, Wageningen Environmental Research / Environmental Risk Assessment Team
In contrast to the individual protection foreseen for humans, under the auspices of the regulatory risk assessments for ecotoxicology, protection of populations is assumed to be of greater relevance than protection of individuals. This adds an extra layer of complexity to the risk assessment, as determination of what effects, and what level of those effects, would result in a population level effect remains a large unknown. As a conservative measure, it is assumed that any effects which would result in massive acute toxicity or effects on reproduction would result in population level effects. Furthermore, conservative assumptions are made for the exposure of the entire population (it is assumed that the entire population will be exposed at the same, high, level. However, while it is assumed that this is a conservative path, it is unlikely to be realistic for most uses being assessed. For that reason, population modelling has been developed in order to try to address a more relevant exposure for a population and determine a more likely population level effect. This presentation will summarize our experiences in evaluating a population model for small mammals and conclude with our vision of the pros and cons of this model as a stand-alone and in the context of the regulatory risk assessment for birds and mammals from pesticides in the EU

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Critical Evaluation of Population Models for the Environmental Risk Assessment of Plant Protection Products
J.M. Becker, Helmholtz Centre for Environmental Research / System Ecotoxicology; T. Jager, DEBtox Research / Dept of Theoretical Biology; S. Kramer-Schadt, Leibniz Institute for Zoo and Wildlife Research / Department of Ecological Dynamics; M. Franz, Leibniz Institute for Zoo and Wildlife Research / Deptartment of Ecological Dynamics; M. Solé, UBA - Federal Environment Agency; S. Duquesne, German Environment Agency; S. Pieper, German Federal Environment Agency UBA / Plant Protection Products; M. Liess, Helmholtz Centre for Environmental Research GmbH - UFZ / System-Ecotoxicology
Individual-based mechanistic effect models are increasingly proposed in higher tier studies for the environmental risk assessment (ERA) of plant protection products (PPP). The models have been often applied to demonstrate that effects in lower tier studies will not translate into unacceptable effects on populations under more realistic conditions. We evaluated seven population models and case studies of their application in ERA from a scientific point of view. The models address the aquatic species *Daphnia magna* (IDamP, Preuss et al. 2009), *Chaoborus crystallinus* (Strauss et al. 2016), *Asellus aquaticus* (MASTEP, Van den Brink et al. 2007), the springtail *Folsomia candida* (SpringSim, Meli et al. 2013) and the small mammals *Apodemus sylvaticus* (Liu et al. 2013), *Microtus agrestis* (ALMaSS, Topping et al. 2012) and *Microtus arvalis* (eVole, Wang 2013). Model mechanisms were scientifically justified. The models address standard focal species in ERA, which are typically less ecologically vulnerable due to short generation time and high reproduction that provide high potential for recovery. The case studies did not consider to which extent the results might cover for additional species, which is not according to the claim of higher-tier studies. A considerable source of uncertainty regarding model predictions was identified because population models represent isolated populations without community context, strictly limiting their applicability in ERA. The models typically predicted fast recovery due to density-regulation and recolonisation, which can be undermined by predation and interspecific competition. Simulating ecological processes that reduce the risk of PPP (recolonisation) without also simulating processes that increase the risk (biotic stressors) may not increase realism but result in unbalanced ERA. An extension to community models would be the next logical step. While the population dynamics without PPP exposure have been validated for most models, more effort should be devoted to test the predictions of PPP effects. The case studies applied models in ERA without assessing to which extent relevant predicted endpoints (extinction risk, recovery time) match real-world data. Environmental properties (landscape composition, farming practices) can significantly affect the model output. We conclude it is crucial to establish specific environmental scenarios and protection goals for higher-tier modeling that can be addressed by modelers and risk assessors.

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Poster spotlight

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Latest Developments and Future Needs for Higher-Tier Studies, Risk Assessment and Risk Management in the Regulation of Biocides, Pesticides and Pharmaceuticals (II)

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Latest developments of guidance and guidance-related documents on exposure assessment for biocides and introduction to the risk assessment tool EUSES 2.2.0
H. Schimmelpfennig, ECHA-European Chemicals Agency / D1 - Biocides; E. Nogueiro, European Chemical Agency ECHA / D1
The aim of the talk is to (1) provide an overview on the latest development of guidance and guidance-related documents and to (2) introduce the new EUSES version 2.2.0. (1) Guidance and guidance-related documents related to exposure assessment like the emission scenarios or emission scenario documents (ESD) are continuously being developed by different parties involved in the biocide assessment (i.e. member state, ECHA or industry). During 2017 and 2018 the focus was on the following items: Revised BPR guidance Volume IV Environment (Part B+C) v2.0 - changes in exposure assessment ($PEC_{initial}$ versus PEC_{TWA}); Revised Emission Scenario Document for PT 6 - In-can preservatives; Revised Emission Scenario Document for PT 14 - Rodenticides; Revised scenarios for PT 18 (Insecticides); WG recommendation on the assessment of precursors of in situ generated active substances; Guidance on aggregated exposure assessment; Single emission scenarios for several product types (PT 3, PT 18, PT 2, PT 4, PT6); Development of core emission scenarios. (2) EUSES is a decision-support instrument for the quantitative assessment of exposure and risks of biocides (and other chemical substances). It is based on the EU Technical Guidance Documents (TGD) (→ Vol. IV Part B for Biocides/REACH R16). Since 2014, JRC is no longer involved in EUSES related legal frameworks and developments/revisions after 2012 were no longer implemented (→ 6 ESDs + several single emission scenarios). EUSES update was initiated by ECHA in 2017 resulting in EUSES version 2.2.0, published in Q1 2019. The focus of the update was on biocides only. The IT technology and User interface remained unchanged. Missing emission scenarios were added in the release module, the release module as such was revised for the already existing emission scenario in EUSES. In addition, direct release was implemented in the Fate and distribution module as well as in the Risk characterisation module. The new SimpleTreat version 4.0 was implemented.

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Could a spatially distributed modelling approach enhance post approval considerations for veterinary medicines?
C. McMillan, G. Hughes, J. Carnall, Cambridge Environmental Assessments
The environmental risk assessment framework adopted in Europe for assessing the surface and groundwater risk from veterinary medicines used to treat livestock follows a tiered approach. The initial exposure assessment is a simplistic approach, with the FOCUS suite of models (FOrum for Co-ordination of pesticide fate models and their USe) often subsequently required for higher tier surface and groundwater refinement. Standard FOCUS scenarios defined within the guidance are intended to represent realistic worst case scenarios for assessing leaching behaviour and surface water risk. However, this approach does not help inform post approval considerations such as identifying the regions most vulnerable to groundwater contamination or surface water risk. In this presentation we consider how this could be achieved for groundwater assessments by conducting a scoping

assessment using the current Okehampton scenario and comparing it to a spatially distributed version of the metaPEARL model (Tiktak et al., 2006). This implementation of the metaPEARL model was underpinned by an environmental database at 1 km resolution describing (i) land cover using the CORINE 2012 dataset (ii) soils data sourced from the EU Soil Data Centre e.g. EFSA spatial dataset v1.1 (Heiderer, 2012) and OCTOP (Jones et al., 2004) and (iii) climate data from WORLDCLIM (Hijmans et al., 2005). In some cases soil properties were modified to represent permanent grassland soils. The spatially distributed PECgw values produced were analysed at a range of spatial scales including NUTS2, Member State, pesticide registration zones defined by directive 1107/2009/EC and climatic zones. The results indicate that the current approach used in the registration process for veterinary medicines masks a wide variation in the risk as predicted by a more detailed, spatially distributed approach. This presentation illustrates how a more spatial approach to the environmental risk assessment of veterinary medicines could help provide clarity on the environmental risk posed by authorised products in regions within Europe, particularly in situations where environmental risks are identified but the veterinary medicine product is approved due to other considerations (e.g. animal welfare). This type of approach could help inform decisions on risk management and facilitate a more targeted approach to ecopharmacovigilance and drinking water protection.

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Addressing the knowledge gap in relation to ecotoxicological impacts of biocidal active substances and environmentally relevant metabolites

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The approval of biocidal active substances (BAS) in agreement with the Biocidal Products Regulation (BPR) requires, as a part of the core data set, single species toxicity data on a number of selected organisms assumed as representative of the natural ecosystems. Such information is also needed for the ecotoxicologically relevant metabolites, which are defined by the ECHA as any minor or major metabolite which poses a comparable or higher hazard than the active substance^[1]. Within the LIFE COMBASE Project, chemoinformatic approaches are being developed for the assessment of the ecotoxicological impacts of BAS and their corresponding environmentally relevant degradation products. With the aim of generating new data to validate developed *in silico* models, four different *in vivo* ecotoxicological studies to aquatic organisms have been carried out including: acute toxicity testing on fish, short-term toxicity testing on *Daphnia magna*, growth inhibition study on algae and inhibition of microbial activity. Fish acute toxicity test has been carried out using *Oncorhynchus mykiss* (rainbow trout) as target species, following the OECD TG 203 whereas assays on *Daphnia*, algae and microorganisms have been carried out in agreement with the OECD TG 202, OECD TG 201 and OECD TG 209 tests, respectively. The present work includes data obtained on three BAS: 2-phenoxyethanol (currently in approval process for Product Types –PTs- 1; 2; & 4 and not authorized for PT13), chloramin B (currently in approval process for PTs 2; 3; 4 & 5) and sodium pyrithione (currently in approval process for PTs 2; 6; 7; 9; 10 & 13 and not authorized for PT3), and also data corresponding to five metabolites: 3-phenoxybenzyl alcohol (metabolite from D-Phenothrin); benzimidazole (from thiabendazole); cyanoacetamide (from DBNPA); benzenesulfonamide (from chloramine B) and cis-2,6-dimethylmorpholine (from fenpropimorph). In general, results obtained are coherent for the different trophic levels considered, although in some cases fish and microorganisms are the most and least sensitive species, respectively. The results obtained allow stablishing a PNEC for these substances which can be used for risk assessment and management purposes. **Acknowledgements:** LIFE-COMBASE project (LIFE15 ENV/ES/000416) <br clear="all" /> [1] REF: Guidance on information requirements – GUIDANCE ON REGULATION (EU) Nº 528/2012 COVERING THE MAKING AVAILABLE ON THE MARKET AND USE OF BIOCIDAL PRODUCTS (BPR) Version 1.0. July 2013 ECHA

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Risk Mitigation Measures for Pesticides in the EU (MAgPIE project) - challenges and recommendations from the workshop experts

A. Alix, Corteva Agrisciences / Risk Management

The MagPIE project initiated in 2012 allowed the development of a toolbox to mitigate environmental risks posed by plant protection products used in agriculture. The proceedings were published in 2017, which propose an inventory of the risk mitigation measures in place in European Member States as well as recommendations to facilitate further development and implementation. These proceedings reflect the view of 95 experts from 24 European countries, together with experts from Switzerland, Norway, the United States of America, the European Commission and the European Food Safety Authority, and representing regulatory agencies in charge of the risk assessment of plant protection products, regulatory authorities in charge of the risk management and decision making, crop protection industry, universities and research institutes, technical institutes involved in the development of risk mitigation tools, or the measurement of their performances, farmers and agriculture advisors, and Non Governmental Organisations. The project allowed to identify the most promising tools for the mitigation of pesticide transfers and possible environmental risks, which gathered risk mitigation measures and tools already impleneted in European countries but also the most promising tools for a short term implementation. The proceedings also discuss the challenges met along the stakeholder chain when implementing, by farmers, regulators, risk assessors, or decision makers. This poster aims at listing these challenges were identified during the MAgPIE workshop together with the recommendations of the expert group to tackle them, including illustrated examples.

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From national to regional risk mitigation measures - opening the toolbox for conditions of use for plant production products

G. Czub, Federal Office of Consumer Protection and Food Safety BVL / Plant Protection Products; C. Kula, Fed. Office for Consumer Protection & Food Safety / Department of Plant Protection Products

In the EU Member States, an authorisation of plant protection products (PPP) is only granted if the risk assessment demonstrates that by application of appropriate mitigation measures the risk for the environment remains acceptable in any of the landscapes in which the product might be used. In general, an authorisation is granted on a national scale in conjunction with risk mitigation measures to be applied independent on the regional conditions. For several reasons it might be worse to consider a revision of this approach: (i) active substances might exhibit characteristics critical only under certain circumstances but otherwise effective and preferable means for the plant protection and agricultural production, (ii) certain regional structures might require specific protection or have characteristics impeding the application of common national mitigation measures, (iii) the number of active substances available in authorised PPP decreases remarkable, hampering an effective pest control and resistance risk management. Regions in focus for specific mitigation measures include, among others, drinking water abstraction areas, sites of high biodiversity value, but also highly trenched areas of small field sizes. For a sustainable plant protection a paradigm shift might be required, allowing for an authorisation (or rejection) of PPP for use in specific regional areas instead of an authorisation on an entire national scale. Challenges of such an approach are, among others, technical prerequisites such as detailed regional data compilations (e.g. GIS-mapping), its consideration in the regulatory risk assessment, the communication of measures and restrictions to the end user and the possibility to control these specific mitigation instruments. Monitoring programmes might become an essential prerequisite in order to evaluate the applicability and effectiveness of regional mitigation measures. Taking into account the current developments and the associated consequences for plant protection, a shift from nationally applied restrictions of use and mitigation measures to restrictions and mitigation measures on a regional scale might be required to maintain a high environmental protection level.

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New Approaches to Environmental Risk Assessment of Veterinary Pharmaceuticals at US Food and Drug Administration's Center for Veterinary Medicine

H. Zahner, E.M. Silberhorn, U.S. Food and Drug Administration / Center for Veterinary Medicine

This presentation will discuss current regulations, guidance, policy, and approaches used at the US Food and Drug Administration's (FDA's) Center for Veterinary Medicine (CVM) to prospectively evaluate the potential for environmental impacts of new animal drugs. Under the National Environmental Policy Act (NEPA), FDA is responsible for determining whether the agency's actions (e.g., approval of a new drug) will result in potential environmental impacts. NEPA provides tools, such as environmental assessments (EA) and categorical exclusions from the requirement to prepare an EA, for evaluating the environmental safety of agency actions. In addition, CVM has published a Guidance for Industry (GFI) with recommendations on how to conduct an EA that was developed and harmonized with regulatory agencies in the European Union and Japan, and has since been adopted by others. This guidance recommends a tiered approach to testing that includes an evaluation of the fate, transport, and effects of animal drugs in the environment, and recommends using a traditional risk quotient approach when assessing risk. However, CVM's GFI is not applicable to the evaluation of some new animal drugs. For example, the current GFI does not provide agency recommendations on how to estimate potential environmental concentrations in soil or water, or on how to evaluate an animal drug that has an unusual route of exposure (e.g., dietary), is known to be toxic at very low concentrations (e.g., endocrine active compounds, parasiticides), those that bioaccumulate in organisms, or those that persist in the environment. Examples of novel approaches used by the animal drug industry and CVM to address current issues will be presented. In addition, CVM's current and future scientific perspectives regarding the EAs for animal drugs will be discussed.

Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring New And Novel Approaches (I)

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Setting the baseline in in vitro bioassays

B. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; F.C. Fischer, Hemholtz Centre for Environmental Research - UFZ / Cell Toxicology; L. Glauch, Helmholtz Center for Environmental Research UFZ / Cell Toxicology; L. Henneberger, Helmholtz centre for environmental research - UFZ / Cell Toxicology; M. Koenig, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; R. Schlichting, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology

Cell-based bioassays have become popular in chemical risk assessment and monitoring of environmental samples. With a suite of simple short-term reporter gene activation assays it is possible to obtain a picture of potential chronic adverse effects and information on the modes of action of chemicals and samples in an adverse outcome pathway framework. Recent advances in high-throughput screening such as the Tox21 initiative and ToxCast have generated a large database of effect concentrations for hundreds of endpoints suitable for various risk assessment applications. Despite the rapid advances in this field, some practical questions remain as well as issues with the interpretation of the effect concentrations derived from concentration-response curves. Robotic pipetting platforms increase throughput and permit decrease in bioassay volumes to as little as a few μL but the trade-off are higher evaporative losses and a reduced applicability domain. We derived a cut-off for volatility, which is actually a cut-off related to the medium-air distribution ratio, that is driven not only by the vapour pressure but also by solubility and the protein- and lipid-water partition constants. Further, many reporter gene assay results are compromised by the so-called cytotoxicity burst, where close to cytotoxicity, various reporter genes are activated in a nonspecific fashion. We have not only improved quantification of cytotoxicity but also experimentally derived baseline toxicity QSARs for eight mammalian cell lines. We concluded that any effect concentration that is higher than the inhibitory concentration for cytotoxicity is likely to be an artefact of the cytotoxicity burst. It is notable that the baseline toxicity QSARs were fairly similarly for all cell lines and mass balance modelling demonstrated that the small differences are related to differences in bioavailability and make up of cells and medium, with virtually constant critical membrane concentration, which are also in the same range as the critical membrane concentrations for narcosis in aquatic species. The developed baseline toxicity QSARs help to design experiments, which should always be run up to baseline toxicity concentrations to assure that no effects are overlooked, and to interpret effect data with respect to the degree of specificity of the effect and for *in-vitro* to *in-vivo* extrapolations. This study is part of the CEFIC ECO36 project.

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Performance of three-dimensional rainbow trout (Oncorhynchus mykiss) hepatocyte spheroids for evaluating biotransformation of pyrene

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Hydrophobic organic compounds have the potential to bioaccumulate in tissue and lipid reservoirs of aquatic organisms such as fish, potentially causing both short and long-term biological effects. The assessment of a compound's potential to bioaccumulate in fish involves its physicochemical properties (e.g. Log K_{ow}) and rate of biotransformation, using conventional animal (*in vivo*) test methods (e.g. OECD TG 305). Due to the ethical and economical concerns, development of non-animal computational (*in silico*) bioaccumulation assessment models (e.g. Quantitative Structure Activity Relationships) for aquatic vertebrates (fish) have been proposed. To improve existing computational predictions, various *in vitro* tests (e.g. S9 fractions and primary hepatocytes) have been proposed for their suitability as alternative bioaccumulation models. One recently developed model, is the three dimensional (3D) hepatic spheroids of rainbow trout (*Oncorhynchus mykiss*). The aim of present study was therefore to evaluate the metabolic competence of 3D-spheroid hepatic cultures of rainbow trout when incubated with the model compound pyrene (PYR). The results were compared with previously published intrinsic clearance (CL) results for S9 and primary hepatocyte assays. In addition, two different sampling procedures, estimating the total- (i) and free (ii) concentration of PYR and its primary metabolite OH-PYR were assessed. Extraction of total (bound and free) PYR (i) suggested that the spheroids had depleted 33% of the added PYR within 4 h of exposure, further metabolizing a total of 85% and 91% after 24 and 30h respectively. However, when applying the second (ii) sampling procedure, a substantial amount (36%) of the PYR was, within 2 h of exposure, bound to the glass wall of the exposure vial. The PYR continued to be bound (33% and 2%) to the glass wall for the duration of the exposure (6 and 30h, respectively). Log-linear formation of OH-PYR was obtained throughout the study, displaying the 3D-spheroids metabolic competence. The two sampling procedures yielded differently measured $CL_{in\ vitro,\ spheroid,}$ where PYR depletion in the cell+media was highly similar to previously published studies using cryopreserved hepatocytes. The 3D-spheroids demonstrated high reproducibility, log-linear biotransformation of PYR and formation of OH-PYR, indicating that 3D-spheroids are highly metabolically comptetent for 30 h or more.

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Integrated alternative approaches for bioaccumulation and toxicokinetics: from in vitro data to in silico models

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Under REACH, more data for chemicals have been assembled than ever before. Unfortunately, the quality of data is not always adequate and, in many cases, critical information, mainly related to toxicokinetic (TK) processes (i.e., biotransformation rates) is not available. Testing programs are expensive and require a high number of animal lives per chemical, which is why REACH advocates *in vitro* assays as a means to reduce animal-testing. Integrated Testing Strategies (ITS) comprise both testing and non-testing methods, such as *in vitro* testing and computational methods that aim to reduce animal testing and optimize intelligent use of available information to improve chemical hazard and risk assessment. The CEFIC LRI ECO44 "Integrating Bioaccumulation Assessment Tools for Mammals (iBAT-Mam)" project's primary objective is the integration of multiple data streams of TK data in a coherent framework for the assessment of bioaccumulation in mammals. Here, more than 30 years of *in vitro* and *in vivo* TK data (rodents and human) are assimilated and critically evaluated. Then *in vitro-in vivo* extrapolation (IVIVE) models and Quantitative Structure-Activity Relationships (QSARs) are developed to maximize the information available from *in vitro* and *in vivo* measurements. In addition, Quantitative Activity-Activity Relationships (QAARs) are used to examine interspecies correlations and predict missing information by extrapolating from one species to another. This presentation will describe on-going methods to address data gaps in biotransformation rates at multiple levels of biological organization through the development of validated QSARs following OECD guidance. Preliminary correlation analysis performed between intrinsic hepatic clearance measured *in vitro* in rodents and humans revealed high interspecies correlation $R^2 > 0.70$. QSAR models were generated for different structural groups to predict the hepatic clearance in human and rodents. All models are stable, robust ($R^2 > 0.70$ and $Q^2_{loo} > 0.65$), externally validated and predictive when they are applied to generate different splitting schemes with particular attention to the structural applicability domain (AD). In conclusion, *in vitro* biotransformation rate data can be obtained to systematically address uncertainty. New QSARs and predictive methods for rates and half-lives are in development within this project.

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Detoxification capacity of zebrafish (Danio rerio) early life stages: Characterization of the glutathione conjugation pathway by targeted proteomics and metabolite analysis

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Zebrafish early life stages offer an important model for the study of disease and chemical risk assessment due to their genetic similarity to humans and the availability of well-established high-throughput techniques for toxicity analysis. However, in order to understand the validity of this model for comprehensive assessment of bioactive compounds, it is important to characterize the biotransformation capacity of zebrafish embryos. Here, we focused on detoxification pathways mediated by glutathione S-transferases (GSTs). This enzyme family plays a major role in phase II biotransformation processes, and GST-catalyzed conjugation reactions are a critical contributor to detoxification and clearance of various intracellular metabolites, but also natural toxins and xenobiotic compounds. First, we developed multiple reaction monitoring (MRM) assays that enable monitoring specific GST isoenzymes and GST classes through a combination of proteotypic peptides and peptides shared within the same class. Our analyses showed that a few representatives of the four GST classes (alpha, mu, pi and rho) are present in zebrafish embryos as early as 4 hours post fertilization (hpf). The majority of GST enzymes, however, only become expressed at 72 hpf, followed by a continuous increase in expression [1]. Next, we studied the biotransformation capacity of the expressed GSTs in zebrafish embryos and early larvae exposed to non-toxic concentrations of the reference substrate 2,4-dinitrochlorobenzene (CDNB). CDNB and its mercapturic acid pathway biotransformation products were measured by LC-MS. In parallel, changes in protein expression of cytosolic GSTs were monitored. Our results demonstrate that early zebrafish life stages are able to perform glutathione conjugation reaction with CDNB and to further process the product within the mercapturic acid pathway. The early expression of a wide range of cytosolic GST classes and the functionality of the mercapturic acid pathway supports the functionality of the zebrafish early life stages as an alternative model in toxicology and chemical hazard assessment. [1] Tierbach A, Groh KJ, Schönenberger R, Schirmer K, Suter MJ-F. 2018. Glutathione S-Transferase Protein Expression in Different Life Stages of Zebrafish (Danio rerio). Toxicological Sciences.162(2): 702–712

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Developing in-silico predictions of toxicity

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Human activities, including manufacturing and pharmaceutical processes, increase the abundance of different toxins. The potential environmental impact of the manufacture and use of pharmaceuticals is still not known, and as the number of compounds in the environment is increasing, not all compounds can be tested. *In–silico* approaches, such as quantitative structure-activity relationships (QSARs) models, can be used to address these issues. This type of models have been used for the prediction of the biological activity, toxicity, and other properties, using the chemical structure of the compounds based on the assumption that the structure of a molecule is responsible for the physical, chemical and biological properties. Zebrafish have been exposed to a variety of toxic compounds, including endocrine disruptors, at different concentrations for up to 96h. A log-logistic model was used to calculate the LC50 and the slope of the dose-response curve of each compound. In addition, the General Unified Threshold (GUTS) model of survival, will also be used in order to validate the LC50 values that are going to be used in the generation of a predicting model. For the generation of the model a list of molecular descriptors was obtained using Dragon 7 software, for over 150 toxic compounds. Descriptors such as ALOGP (degree of hydrophobicity), spectral moments and autocorrelation of topological structure (ATS) descriptors, increase the accuracy of our predictive models. Heart-rate data, for up to 96 hours, will be used to generate a model that can predict the heart-rate of the zebrafish, based again on the structure of the molecule. The quantitative structure-activity (QSAR) models will be integrated into the web application, which can take any compound and will be able to provide the user with the predicted LC50 and the slope values and the expected change in heart-rate. The application will help predicting missing experimental data and prioritize chemicals for toxicity testing, which can save time, effort and reduce the number of experimental animals.

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QSARs for thiochemicals - a case study of using alternative information for REACH registrations

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A case study on acute aquatic toxicity of thiochemicals is presented to show the possibilities and limitations of filling data gaps with alternative information in accordance with the requirements of REACH [1]. Experimental studies with fish, daphnia and algae are available for several case study chemicals. It is the objective of this study to extract as much information as possible from the available experiments to estimate required data by QSARs, read-across and trend analyses. Thiochemicals are considered to be toxic with an unspecific reactive mode of action (MOA) causing so-called excess toxicity, i.e. the effects are much higher than estimated from baseline QSARs. Differences in toxicity between the groups of thiochemicals are thought to be due to differences in reactivity of the respective sulfur moiety, i.e. toxicodynamic differences. Within each group the thiochemicals share the same reactivity due to the identical sulfur function, but are different with regard to partitioning between biophases related to increasing aliphatic chain length, i.e. toxicokinetic differences. Due to the toxicodynamic and toxicokinetic differences, QSARs, read-across and trend analyses are limited to thiochemicals within the same group. Scientifically valid QSARs for thiochemicals are not available and the present data base is too small to derive new QSAR models that fulfil the OECD principles for the scientific validity of QSARs. Most of the 36 data gaps for 16 thiochemicals to be registered by 2018 were closed by estimates using trend analyses and read-across for thiochemicals within the same group, e.g., thioglycolates or mercaptopropionates. The testing strategies to fill remaining data gaps recommend tests with algae (6 substances) and daphnia (6 substances). Only for two substances experimental (limit) fish studies are recommended. Thus, a substantial (>60%) reduction of animal experiments is possible. [1] Ahlers J, Nendza M, Schwartz D. 2019. Environmental hazard and risk assessment of thiochemicals. Application of integrated testing and intelligent assessment strategies (ITS) to fulfil the REACH requirements for aquatic toxicity. Chemosphere 214:480-490.

Natural Resources in LCA: Extraction, Processing and Dissipation of Metals, Minerals and Plastics (I)

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Abiotic depletion potentials (ADPs) for elements revisited

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In 1995 the original method for calculating the Abiotic Depletion Potentials (ADPs) was published [1]. The ADP of resource i was defined as the ratio of P_i and the square of R_i for resource divided by the same ratio for a reference resource (Antimony (Sb)); hereby P_i represent the annual production (kg/yr) of resource , and R_i its ultimate (crustal-content based) reserve (kg). In 2002 ADPs were updated based on most recent USGS annual production data. In addition, the ADP was sub-divided into two categories and two sets of ADPs: $ADP_{fossil\ fuels}$ and $ADP_{elements}$. Since then, the $ADP_{elements}$ has not changed despite ongoing annual updates of production data and also a few updates of crustal-content data that constitute the basis of the ultimate reserves. Moreover, it was known that the coverage of elements by ADPs was incomplete. These three shortcomings can affect relative ranking of elementary resources and also normalized indicator results for ADP-based abiotic resource depletion. This was realized during the European Commission Product Environmental Footprint pilot project modeling process in 2018, where it was realized that incomplete ADP normalization was incorrectly dominating single score results. On top of this, dealing with annually changing production may need new calculation procedures for the ADP. The present study describes an effort to update the data (P_i and R_i), but with wider ramifications, including an analysis of the changes over time, the resulting variations and a proposal for a more robust updating procedure.

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Assessment of resource uses with dynamic stock models: ADP as a marginal approach from Hubbert peak theory

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In LCIA, the resource issue is still debated and remains the least consensual area of concern. The currently popular assessment models for resource depletion are based on “heuristic” considerations. This presentation deals with the use of a stock dynamic model to address the resource depletion by marginal approach. Based on empirical data simulating the evolution of the exploitation rate, stock dynamics models have been proposed in the literature. It is thus possible to define a mathematical relationship linking the human intervention (the resource extraction) to the impact (the stock depletion). The partial derivative is then used expressing the marginal change and determining the Characterization factors (CFs). This approach was used on fish stocks, and the similarities of the result with the Abiotic Depletion Potential (ADP) proposed by Guinée and Heijungs in 1995 lead us to look at the parallel we can draw. The fish population dynamics model is based on the very common logistic function. This is also the relation used in the Hubbert peak theory. We use the shape of the model to describe the current depleted resource fraction. This fraction equals to zero for a never exploited resource and tends towards one with the depletion. The use of partial derivative therefore makes it possible to define the CF of the resource depletion on the basis of the Hubbert theory. The difference between ADP and Hubbert-based CFs lies only in the constant part of the equations with a fixed (antimony-based) unit conversion factor for ADP and a resource-specific parameter for Hubbert-based CFs. We show here that the original ADP is a very good estimator for the CF derived as a marginal depletion characterization factor from the Hubbert curve with an high correlation (r=0.99 with p-value

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Regionalized Global Life Cycle Impact Assessment of Natural Resource Extraction and Processing

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Over the past decades, the continuous growth in human population and economic welfare has increased the pressure on natural resources and the environment to an extent that is not sustainable. Thus, decoupling the environmental impacts from economic growth has been defined as a policy objective by the United Nations Environment Program. For this purpose, detailed assessments that quantify the impacts of global resource production from a life-cycle perspective are crucial, but are lacking so far. Here, we provide a globally regionalized impact assessment of multiple environmental and socio-economic indicators, such as water stress, land-use related biodiversity loss, health impacts due to particulate matter emissions (PM health impacts), together with climate change impacts, value added, compensation of employment, workforce and risks for employment. Further, we developed a new method to allocate these impacts to the extraction and processing of resources, called resource-related impacts in the following. This allowed us to assess the temporal development of multiple resource-related footprint indicators between 1995–2011 for the entire globe and for single nations. The regionalized assessment showed, that all nations have achieved a decoupling of the environmental impacts of their economic growth, but most nations did not achieve a decoupling from their population growth, meaning that the per capita impacts have increased over the past. Further, we discuss the global trade pattern of resource-related impacts over three stages of the supply chain to identify hotspots of production, consumption, and trade of the environmental impacts of key resource types. Results of this regionalized global impact assessment are important to assess progress towards national and global environmental policy targets, and serve as a basis to establish policy options for a more sustainable production of resources. The method developed in this study can be applied on any industrial sector(s) and region of interest to trace its regionalized impacts over the global supply chain from a production, sector output as well as final demand perspective.

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Extending the Geopolitical Supply Risk method: Potential for recycling as a risk mitigation strategy

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Criticality indicators are being used to inform management decisions on supply risk and use of critical raw materials needed for emerging technologies. Improving and promoting recycling processes is suggested as one of the strategies to support the transition to a circular economy and address the concerns linked to supply risk. Existing methods to assess supply risk of materials based on geopolitical considerations do not consider the potential effects of recycling. This can be overcome by enhancing the Geopolitical Supply Risk (GeoPolRisk) methodology, which can be integrated into a Life Cycle Impact Assessment (LCIA) characterization model. The aim of this study is to provide a measure for the potential reduction of supply risk that can be achieved with domestic recycling. The GeoPolRisk method measures the supply risk of a commodity based on the supply mix to a country and the global production shares of suppliers. A previously published study was used as a baseline for further assessment of potential supply risk reduction attributable to domestic recycling from the perspective of the European Union (EU-27) including recycling indicators made available by the European Commission. A comparison is made between the previous GeoPolRisk results and the updated values after the inclusion of recycling considerations. Two scenarios were studied: a best case scenario (BCS) that assumes a preference for low-risk suppliers and a worst case scenario (WCS) that results in the selection of high-risk suppliers. Independent simulations were developed for the analyzed materials. With the inclusion of recycling considerations into the GeoPolRisk methodology it is possible to obtain a measure of potential supply risk reduction. A powerful feature of this enhanced methodology is the provision of a range that captures the effect of domestic recycling on the supply risk of materials under different scenarios that depend on the purchase decisions made by buyers of raw materials. A novel tool integrated into a LCIA characterization model is provided to help guide decisions regarding the adoption of recycling technologies and the selection of suppliers for product systems. Further application of the methodology can also provide organizations with indicators to promote recycling practices on a national and regional level in order to support the transition towards a circular economy.

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Metal and mineral resources in LCIA - The SUPRIM project

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Over the past two decades, life cycle impact assessment methods have received extensive attention and developed into mature methods mostly reflecting the state of the art in the relevant scientific field. Despite these achievements and harmonization efforts by the UNEP-SETAC Task Force on natural resources, there is still a lack of consensus on how to assess abiotic resource use in life cycle impact assessment (LCIA). This lack of a broadly accepted method, likely attributable to the lack of a common perspective on resource use and a common understanding of the potential problem(s) related to the use of resources, was the starting point of the SUPRIM project. The aim of SUPRIM is to obtain an understanding of different stakeholders' views and concerns regarding potential issues which result from the use of resources. The gained insights will be provided in the form of a structured overview of those different views, which will then be used as a basis for further method development. This is being achieved by 'taking a step back' to a structured discussion about potential problems with resource use, and different motivations behind resource management concepts. The project focuses on impacts which occur directly from the use of abiotic resources - more specifically, abiotic resources such as minerals, metals, and natural materials - e.g. sand or natural stone. As one of its first activities, the SUPRIM consortium developed a framework for a structured discussion of different perspectives on resource use. The framework consists of an overarching perspective, a conceptual level ("Modelling Concept"), and a practical implementation level. The framework was first used as basis for a workshop discussion with external stakeholders. Prior to the workshop, the framework was introduced to the external stakeholders by way of a discussion paper including a number of pre-defined perspectives for discussion. Next, the workshop results were adopted as basis to propose best modelling options matching the perspectives and problems identified and prioritized by the workshop participants. The SUPRIM work is currently still ongoing but expected to be finalized in Spring 2019, and we aim to present first details of preferred methods at the conference.

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Development of a conceptual framework and its associated indicator to take the dissipation of non-energetic abiotic resources into account within Life Cycle Assessment (LCA)

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Recent developments for the Natural Resources Area of Protection (AoP) in Life Cycle Assessment (LCA) have put current methods tackling resource use under review by many (e.g. Drielsma et al., 2016; Klinglmair, Sala, & Brandão, 2014). It has been proposed that the safeguard subject for mineral resources is their capacity to fulfill provisioning functions for humans (materials, energy, food, space, etc.) (perspective 2 from Dewulf et al. (2015)). Therefore the damage on resources should be quantified as the reduction or loss of this capacity caused by human activity. Dissipation has been identified as a relevant approach for this perspective compared to methods based on resources depletion (Zampori & Sala, 2017). It allows a better identification of where resource are lost (dissipated), including within the technosphere, and as a consequence lose their potential value for human use. However, no consistent method has been developed to include dissipation within LCA thus far. In this regard, we propose to build a conceptual framework to include dissipation of metals and minerals within LCA. "Key elements", such as dissipation occurring during extraction, production and use, quality limits (e.g. physicochemical limits, including thermodynamics, after which the resource will be permanently lost as an emission or as a final stock within mine tailings or end of life discharge), resource occupation in the technosphere, recycling rates and quality loss are defined in order to make this approach functional. Approaches to measure or evaluate such key elements, and the way their implementation within Life Cycle Inventories (LCI) or Life Cycle Impact Assessment (LCIA) can be done, are presented. Feasibility of the implementation of these elements within the LCA methodology is evaluated and planned future works based on this initial framework are presented. The developed framework represents the first steps towards evaluating dissipation rather than depletion of mineral resources in an LCA context, with an appropriate inventory and impact method. This will lead to the possibility to distinguish between dissipated minerals and recycled ones, which are kept within circular economy and contribute to resource efficiency, and help to support decision-making based on such criteria.

Target and NonTarget Mass Spectrometry for Human and Environmental Exposure Assessment (II)

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High resolution mass spectrometry to investigate the fate of pesticides during microalgae-based bioremediation treatment of water

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Pesticides are substances highly used for many purposes worldwide. After their use, they may end up in the aquatic environment, representing a risk to exposed organisms. To reduce their environmental impact, it is necessary to develop technologies capable of removing them before their release into the environment or from pesticide-containing waters. In this context, the objective of this work was to study a bioremediation technology based on microalgae to remove pesticides from water. For this, microalgae-based degradation studies were carried out at lab-scale with three different pesticides: acetamiprid, propanil and bentazone. All experiments were performed in triplicate, and controls were used to quantify adsorption and photodegradation. Pesticide degradation was monitored at time zero, and after 2 and 7 days of treatment using liquid chromatography coupled to heated electrospray-high resolution mass spectrometry (LC-HESI-HRMS) detection using a hybrid quadrupole-Orbitrap (Q-Exactive) analyzer. Mass acquistion was carried out in full scan and data-dependent MS/MS mode. This type of analytical approach also allowed the investigation of the degradation products (TPs) formed during the process. The results obtained showed that propanil and acetamiprid were degraded in the presence of the microalgae. Propanil was fully degradated after 2 days of treatment, whereas complete degradation of acetamiprid required 7 days. On the contrary, no degradation at all was observed for bentazone. As for the formation of TPs during the process, only one TP could be identified so far. This TP was found in acetamiprid containing waters and its accurate mass and assigned molecular structure corresponds tentatively to the hydroxylation of the parent compound. Additional chromatographic peaks present in t=2 days and t=7 days samples are under evaluation. Identification of additional TPs would assist in describing microalgae biodegradation mechanisms. *Acknowledgement* - This work has received funding from the Government of Catalonia (2017 SGR 01404 and 2017 SGR 00014), the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the project BECAS (grant number CTM2016-75587-C2-2-R), and the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 727450. This presentation only reflects the authors' views and the Commission is not responsible for any use that may be made of the information it contains.

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Pharmaceuticals and their Transformation Products in Hospital Wastewater and Conventional Wastewater Treatment Plants

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Pharmaceuticals (pharma) are extensively used and introduced into our wastewater where inadequate removal leads to release into surface waters. Hospitals have been identified as a major point-source for pharma entering municipal sewers and waterways, and rural hospitals are of concern as subsequent treatment may be carried out with smaller, less advanced wastewater treatment plants (WWTPs). The importance of characterising pharma degradation products is becoming more evident as there is little information available on their presence, behaviour and biological activity. Characterising the effect hospitals have on pharma pollution in municipal wastewater and understanding pharma degradation behaviour and removal in WWTPs are necessary to inform future decision making regarding required infrastructure upgrades to mitigate risks to the environment. Samples were collected from Caithness General Hospital (Wick, UK) and from separate stages of the receiving WWTP. Liquid chromatography-tandem mass spectrometry was used for quantitative analysis of eight compounds: paracetamol, diclofenac and ibuprofen (analgesics/anti-inflammatories), clarithromycin and trimethoprim (antibiotics), carbamazepine and fluoxetine (psychiatric drugs) and 17α-ethynylestradiol (synthetic hormone). Pharma concentrations were in the mid-high ng/L range, with the analgesics/anti-inflammatories and antibiotics detected in the highest concentrations. High resolution mass spectrometry was used for suspect-screening and non-target analysis to identify pharma degradation products and elucidate transformation behaviour within the WWTP. Removal efficiency was calculated from the influent and effluent concentrations, and indicated that the conventional treatment techniques employed are ineffective for pharma, most notably antibiotics. A risk assessment was carried out by comparing observed concentrations, eco-toxicity data and prescription rates in Scotland. This suggested that the compounds of most environmental concern are paracetamol, fluoxetine, diclofenac, clarithromycin and 17α-ethynylestradiol in our study, and source control may be the best preventative measure to reduce burdens on WWTPs and protect water quality. The distribution and degradation of these compounds in WWTPs and effluent-receiving surface water should be further investigated.

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Occurrence and seasonal variation of pharmaceutical compounds from four therapeutical classes in the influents of two Portuguese wastewater treatment plants

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Pharmaceutical compounds (PhCs) are one of the major classes of emerging contaminants due to their intrinsic biological activity. Their presence, even in trace concentrations, raise the possibility of causing adverse effects on both aquatic ecosystems and humans due to chronic exposure. Although the occurrence of PhCs in the urban water cycle has been extensively studied, the understanding of the effects of conventional wastewater treatment processes on PhCs removal is still scarce. Wastewater treatment plants (WWTPs) are crucial barriers against PhCs, with their efficiency depending of several factors such as contaminants psychochemical properties, climate conditions, technology, operational conditions and contaminants concentration in the raw influents (WWI). Therefore, a detailed characterization of WWIs load is important and mandatory towards improving WWTP efficiency. In this work, a SPE-LC-MS/MS method was optimized and validated for the measurement of 24 PhCs (10 therapeutical classes) in WWIs from two Portuguese WWTPs: Beirolas and Faro Nw. Under project LIFE IMPETUS, a total of 41 sampling campaigns were performed between September 2016 and July 2018. These two WWTPs showed similar concentration PhCs profile, being the most representative therapeutical classes analgesics (acetaminophen, APAP), psychostimulants (caffeine, CAF), NSAIDs (diclofenac, DCF, ibuprofen, IBUP, and naproxen, NPX) and antibiotics (erythromycin, ERT, sulfadiazine, SDZ, sulfapyridine, SPD, and sulfamethoxazole, SMX). Based on these results, these therapeutical classes were the main focus of this work. Regarding seasonal behavior in Beirolas WWIs, median concentrations varied between 1.6 μg/L (antibiotics, winter 2017) and 87.2 μg/L (analgesics, winter 2018). Seasonal variations were observed in analgesics (APAP), antibiotics (mainly due to SDZ) and, in less extent, NSAIDs (mainly IBUP), particularly in autumn and winter seasons. Regarding Faro Nw WWIs, median concentrations varied between 0.95 μg/L (antibiotics, spring 2018) and 78.3 μg/L (analgesics, winter 2018). Seasonal behavior was subtler in Faro Nw, possibly due to milder climate conditions, and the variations were observed in analgesics (APAP) and, in less extent, antibiotics. For both WWIs, psychostimulants showed no clear seasonal variations. Overall, this approach enables a comprehensive overview of PhCs profile in WWIs, and consequently, giving relevant information towards improving the WWTPs removal efficiency.

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Beyond freshwater ecosystems: bioaccumulation of emerging contaminants in plants growth in Saudi Arabia oasis impacted by wastewater

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There is increasing concern on food safety because of environmental pollution. Soil pollution with anthropogenic emerging contaminants (CECs) coming from urban areas, industry or agriculturel such as pesticide, fertilizers, pharmaceuticals, personal care products, illicit drugs has received much attention in recent year. CECs in soils can not only be biodegraded, but also bioaccumulated and biotransformed by plants, and pose toxicity beyond certain limit. This study aims at assessing the problem of environmental contamination in two areas of Saudi Arabia (Al-Hayer and Al-Asfar) heavily affected by the use of non-conventional water resources (mostly treated wastewater) through the assessment of these compounds in water, soil and sediments. Furthermore, the potential uptake and bioaccumulation of those CECs found in soil was also evaluated. To this end, pharmaceuticals (carbamazepine, ibuprofen, gemfibrozil, etc…), personal care products (parabens, bisphenol A, etc…) and pesticides (diazinon, chlorpyriphos, etc..) were determined using extraction with methanol and a solid-phase clean-up (SPE) followed by liquid chromatography-tandem mass spectrometry with a triple quadrupole (LC-MS/MS). Microplastics were also determined in water samples by filtration of 20 L though a 150 μm net. Once isolated in the filters, several treatments with oxidants and counting visually using microscope. The results pointed out the widespread contamination of water, soil and sediments by these CECs, including microplastics. The assessment of crops and other plant material showed that several CECs, including six pharmaceuticals (atenolol, caffeine, carbamazepine and its metabolites 10,11-epoxycarbamazepine, gemfibrozil, and naproxen) and seven pesticides (acetamiprid, atrazine deethyl, azoxystrobin, bupirimate, diazinon, malathion, pirimicarb and some of their metabolites) were detected in plants. Up to our knowledge, this is one of the pioneering studies demonstrating the CECs' accumulation in crops irrigated with treated wastewater under non-controlled environmental conditions. *Acknowledgement* – The authors thanks the financial support from the Distinguished Scientist Fellowship Program (DSFP) from King Saud University, Saudi Arabia. The mass spectrometry section of the SCSIE of the Universitat de València is also acknowledge for the help R. Álvarez acknowledge the Spanish Ministry of Science, Innovation and Universities for his FPI grant BES-2016-078612.

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Determination of personal care products in wild fish bile using hybrid solvent precipitation and dispersive solid phase extraction cleanup with UPLC-MS/MS and GC-MS analysis

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Personal care products (PCPs) are ubiquitous in aquatic environments owing to the continuous discharge of domestic wastewater from highly urbanized regions. These PCPs can be adsorbed by wild fish and thereafter usually enter the bile of the fish through biliary excretion. In this study, a quick and sensitive method based on a combination of hybrid solvent precipitation and dispersive solid phase extraction (d-SPE) purification was developed to simultaneously extract and detect 24 PCPs, namely, 16 biocides, 4 synthetic musks, and 4 benzotriazoles, from wild fish bile. Hybrid precipitation on solid phase extraction (SPE) tubes was applied to remove phospholipids and proteins, and a d-SPE procedure was used for further purification. The extraction solvents for the hybrid precipitation/SPE tubes and d-SPE materials were optimized. The method

performances for bile samples both with and without enzyme hydrolysis using β-glucuronidase/aryl-sulfatase were validated. The 24 PCPs in fish bile were spiked with standard concentrations of 10 ng/mL, 20 ng/mL, 100 ng/mL, and 200 ng/mL to evaluate recoveries, which ranged from 70–120% for 16, 16, 22, and 21 analytes with hydrolysis, respectively, and 70–120% for 14, 15, 23, and 23 analytes without hydrolysis, respectively. The quantification limits for target PCPs were in the range 0.26–6.45 ng/mL [excluding musk xylene (MX) and musk ketone (MK)] and 0.20–8.62 ng/mL (excluding MX and MK) for bile samples with and without enzyme hydrolysis, respectively. After enzyme hydrolysis, 12 PCPs were detected in bile from wild fish collected from the Yangtze River, with a maximum detected concentration of 460 ng/mL, for triclosan (TCS). The hydrolysis reaction indicated that high percentages of glucuronide and sulfate metabolites for some PCPs, four parabens, and TCS, existed in the bile.

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Using column chromatography to assess mobility of natural toxins in the aquatic environment
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Over 34% of recently evaluated plant secondary metabolites (natural toxins) were found to fulfil the criteria for aquatic persistency, mobility and toxicity based on predicted property data.[1,2] However, as prediction tools show limited applicability,[3] experimental evaluation of the organic carbon-normalized sorption coefficient (K_{oc}) as a primary mobility parameter is a crucial step to determine if natural toxins end up in potential drinking water abstraction sites. Column chromatography was applied in the systematic evaluation of sorption behavior of a large diverse set of natural toxins to organic matter. Experiments were generally performed as described previously.[3] However, in this study the method was applied to a substantially larger set of compounds and experimental conditions varied to study effects of e.g., temperature and pH on K_{oc}. In addition, method performance parameters were evaluated to ensure method applicability. The case of natural toxins shows that column chromatography is a suitable method for quantitative, reproducible analysis of sorption to organic matter. It is generally applicable to large sets of mobile compounds (log K_{oc} between 0.5 and 3.5). According to the obtained data, most analyzed phytotoxins can be categorized as mobile in the aquatic environment with log K_{oc} values well below the defined cut-off criterion for mobility of log K_{oc} = 4.5.[2] Many toxins are also ionizable and appear in the more water-soluble cationic form in the environmentally relevant pH range and thus show even higher mobility. The very short analysis time and little material requirements easily allow systematic investigations of differing influences on sorption. Thus, detailed mechanistic insights are gained that are of great value for understanding transport and fate processes in the environment. Applying the method to other different sorbents in the future (e.g., minerals, activated carbon) will help to determine their individual contribution to the natural toxins' overall mobility on their path from source to tap (drinking water).
References: 1) Günthardt, et al; **2018**. *J. Agric. Food. Chem.* 66 (29), 7577-7588. 2) Arp, et al; **2017**. *Environ. Sci. Process Impacts* 19, 939-955. 3) Schenzel, et al; **2012**. *Environ. Sci. Technol.* 46 (11), 6118-26.

Your Science or my Science? When the Sides taken on a Research Topic are Impacted by Different Philosophical Positions and Values (I)

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The Role of Philosophical Positions and Values in Scientific Controversies
K. Elliott, Michigan State University / Lyman Briggs College
Scientific controversies often stem from underlying philosophical disagreements. This talk will provide a starting point for the SETAC Special Session, "Your Science or My Science? When the Sides Taken on a Research Topic Are Impacted by Different Philosophical Positions and Values," by clarifying three major ways in which philosophical positions and values can impact scientific controversies. First, those involved in scientific controversies may hold different background assumptions, which result in differing interpretations of the available evidence. For example, recent controversies about endocrine-disrupting chemicals like bisphenol A (BPA) may stem in part from differing assumptions about how to interpret apparent evidence of effects at low doses that are not seen at higher doses. Second, controversies can stem from disagreements about levels or standards of evidence. For example, conflicting views about the toxicity of glyphosate appear to stem at least in part from disagreements about whether particular kinds of studies and trends in data provide sufficient evidence for its carcinogenicity. Third, controversies may stem in part from different ways of framing a problem situation. For example, conflicting views about the acceptability of genetically engineered crops may arise when some analysts are focused on human health effects while others are considering broader social impacts and alternatives. The talk will conclude by discussing ways of addressing these philosophical positions and values in a fruitful manner, both by communicating about them more effectively and by fostering forums in which they can be brought to light.

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The use of pesticides should be critically considered: the emblematic case of neonicotinoids
J. Bonmatin, Centre National de la Recherche Scientifique
Research on pesticides and their impacts has increased in recent decades because of pollution and impacts generated by pesticides for the environment. Experimental data can be interpreted differently, depending on experimental design, sources (for example, unpublished studies and/or peer-reviewed studies), values and conflicts of interest. This can then influence decisions made on a scientific basis, or simply reduce the credibility of science. Is destroying biodiversity and ecosystems a sustainable strategy? Because research on neonicotinoid insecticides is one of the best documented, because these relatively new insecticides are used prophylactically (preventive treatment) and not as a last resort, neonicotinoids illustrate the issue of mass use of pesticides as opposed to sustainability. Science is often a contradictory process where data must be accumulated before a general consensus can be reached. Several meta-analysis are presented on these insecticides. They illustrate the impacts of neonicotinoids on biodiversity and ecosystem services. Paradoxically, these ecosystem services include the services needed for agriculture (for example, soil quality and pollination). Therefore, the current use of these pesticides needs to be critically examined. The assessment should not be limited to private and short-term benefits, but also to a more comprehensive approach which includes increasing pest resistance, limited resilience of the nature, and quality and richness of our environment. Agronomic alternatives must be favoured because they have proved their effectiveness in a vast majority of cases. In addition, it seems increasingly risky to claim that what destroys biodiversity would have no effect on our health.

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Principles to enable farm productivity and conservation through modern crop protection
J.F. Gonzalez-Valero, Syngenta Crop Protection AG
Farmers today face many challenges from climate change, soil erosion and biodiversity loss to changing consumer expectations and views on agricultural technology. In particular the use of pesticides to protect crop quality, safety and productivity are challenged by many. There is an undeniable demand for a shift in crop protection, and a clear call for innovation and more action to address these challenges in ways where everybody wins – from growers to consumers and the environment. Arguably, crop protection products regulation has the highest levels of consumer and environmental safety and it has constantly evolved for more precautionary measure. Environmental Toxicology Science has evolved the safety of pesticides and the role they play in protecting food quality and safety. However, there is a strong believe that technology in agriculture is not needed and hence any safety and risk assessment will biased. At the same time, there is no agriculture without crop protection, be it mechanical, chemical- or biological-designed molecule based, or build in through genetics. All these technologies need to follow the same principles in order to be safe for humans and environment and to protect food quality and quantity: 1) provide solutions for safe and nutritious food, feed, fibers and fuels; 2) Improve soil health for climate-resilient agriculture; 3) Enhance human health and well-being; 4) Protect natural resources; 5) be developed in open communication and partnership to increase the shared value.

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Promoting sustainable use of pesticides and innovation: the Finnish experience
T. Jern, Ministry of Agriculture and Forestry / Department of Food
Finland has a long history of sustainable use of pesticides. The Finnish authorization system has been strict and many old pesticides were phased out already in the eighties and in the nineties. We have statistics on pesticide sales since the early fifties. We have always had a low use of plant protection products and a low occurrence of pesticide residues, both in vegetable products and in surface and ground water. Our first action plan for a sustainable use of pesticides was, published in 2011. We focused on training and information. Our goal was to reduce the health risks from pesticide use through maintaining the good situation with pesticide residues and reducing the environmental risks of pesticide use by developing and steering pesticide use, so that the risk to the environment and to nature would be small. This included developing measures for water protection, promoting a more diverse crop rotation and fostering better practices in handling and storage of pesticides and remnants. We built a training system, which was voluntary for the users of plant protection products. The exam to show that the person really mastered the subject was compulsory. This way a professional user could acquire his/her knowledge either by attending a training course or in another way. The competent authority approved the providers of training and the exam organizers. The second great challenge was to renew the system for testing of application equipment. The first action plan was analyzed in 2017 and in 2018 our second action plan was published. The new program has the same goal – to reduce the risks from pesticide use - but it is more detailed, more ambitious and contains quantitative or qualitative goals. The new plan includes clear actions but also

reviews or assessments, that have to be done to clarify what sort of action needs to be taken. Both for the first and the actual action plan we have sought to involve as large an amount of stakeholders as possible to get them involved and committed to the work. By getting people involved, we hope to see a good commitment to the work, as it is our shared responsibility to achieve a sustainable use of pesticides.

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Discussion with presenters panel and audience

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Biogeochemistry and Fate of Organic Pollutants in Aquatic Systems (II)

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Determination of cyclic volatile methylsiloxanes in German wild fish via a GC-ICP-MS/MS method

G. Radermacher, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Environmental Specimen Bank and Elemental Analysis; H. Ruedel, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Department of Environmental Monitoring; J. Koschorreck, Umweltbundesamt

Keywords: methylsiloxanes, cVMS, fish, retrospective monitoring Cyclic volatile methylsiloxanes (cVMS) are widely applied industrially produced chemicals. For example, cVMS are ingredients in personal care and household products. In addition, they are used as intermediates for silicon polymers. The general structure is a ring built of alternating oxygen and silicon atoms which are substituted with two methyl groups at each silicon atom. Due to their physical and chemical properties (e.g. high hydrophobicity, vapour pressures and Henry's Law constants, relatively long modelled and measured half-lives), cVMS have the potential for long-range atmospheric transport, environmental persistence and bioaccumulation in food webs. It has been shown that cVMS octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) are present in freshwater fish from several countries (e.g. Canada, Norway and Sweden). In 2018, D4, D5 and D6 were listed as Substances of Very High Concern under the EU REACH regulation. Hence, the target of this study was to investigate the occurrence and distribution of D4, D5 and D6 in fillets of German freshwater (bream) and marine (eelpout) fish from major German rivers and the North and Baltic Sea, respectively. Samples of the German Environmental Specimen Bank (ESB) provide the opportunity to analyse time series. First, a solid/liquid extraction method with acetonitrile and hexane mixture was performed. Then, speciation and quantification of cVMS was carried out with a new GC-ICP-MS/MS coupling method. The application of the triple quadrupole technique allows the elimination of interferences, such as CO^+ or N_2^+ ions, on the main silicon isotope (m/z=28) by the use of hydrogen as reaction gas. D5 was found in all samples from the riverine sampling sites. Furthermore, the results show that D5 concentrations were clearly higher than the observed D4 and D6 concentrations. In total, the highest cVMS burdens were determined in samples from the river Saar. In contrast, at ESB reference sampling site Lake Belau as well as in eelpout muscle tissue from the North and Baltic Sea no cVMS were found.

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Analysis and occurrence of organophosphate esters in fish

L. Han, China Agricultural University / College of Science; Y. Sapozhnikova, U.S. Food and Drug Administration / ARS

Organophosphate esters (OPEs) are high volume production chemicals used as flame retardants and plasticizers in many consumer and commercial products. OPEs have been reported in the environmental samples, *i.e.* indoor dust, air, wastewater, marine and river water, ground water, soils and sediments. Most published reports on OPEs' occurrence thus far focused on their levels in air, water, soil, sediments, biota, and humans, and several recent publications emerged on OPEs' occurrence in foods. The goal of this study was to develop a fast and simple method for analysis of the fourteen most commonly used OPEs in fish and apply the developed method for the analysis of fish samples from the USA markets to generate data on their occurrence. Fourteen OPEs selected in this study were: trimethyl phosphate (TMP), triethyl phosphate (TEP), tri-n-butyl phosphate (TnBP), tris (2-chloroethyl) phosphate (TCEP), tris (2-chloro-isopropyl) phosphate (TCPP), tris (1,3-dichloro-2-propyl) phosphate (TDCPP), cresyl diphenyl phosphate (CDPP), tri-cresyl phosphate (TCrP), tri-propyl phosphate (TPrP), tri-phenyl phosphate (TPP), 2-ethyl-hexyl-diphenyl phosphate (EHDPP), tris (2-butoxyehtyl) phosphate (TBEP), tris (2-ethylhexyl) phosphate (TEHP); tri-isobutyl phosphate (TiBP). The developed method was based on QuEChERS extraction, automated mini-SPE cleanup, and UHPLC- and fast low pressure (LP)GC-MS/MS analyses. 14 OPEs were analyzed by UHPLC- and 8 OPEs were analyzed by LPGC-MS/MS. Two isotopically labeled internal standards: TPP-d_{15} and TCEP-d_{12} were used. Method detection limits were 0.5 - 1 ng/g, and OPEs' ubiquitous presence in laboratory environment and materials presented a challenge to their accurate quantitation at levels below 1 ng/g, especially in LC- vs. GC-MS/MS analysis. The method was validated at four spiking levels (5, 10, 20 and 40 ng/g), and satisfactory recoveries (70-120%) and RSDs (< 20%) were achieved for 13 OPEs. The validated method was applied for analysis of different fish samples to generate needed data on OPEs occurrence in fish. TCPP was found in flounder, monk fish and catfish in concentrations 0.3-8 ng/g. More data on occurrence of these emerging contaminants are needed to advance our understanding of their potential risks and aid in future risk assessments and regulations.

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Tracing microbial turnover of pesticides in water-sediment with stable isotope probing

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Pesticides and their transformation products are frequently detected in freshwater sedimentary environments, but there are no comprehensive studies on their behaviour in these systems. Microorganisms can use C and N from a pesticide to synthesize their biomass compounds, e.g. amino acids. Their biomass compounds after the death can be stabilized in the organic matter of sediment forming harmless biogenic non-extractable residues (bioNER). The conversion of a pesticide to carbon dioxide and bioNER leads to their ultimate detoxification. The objective of this study was therefore to investigate the turnover of model pesticides (glyphosate and metamitron) in the water-sediment systems with a particular focus on its biodegradation pattern and bioNER formation using the ^{13}C and ^{15}N-labeling approach. Mineralization in water-sediment was much higher (55.7% of $^{13}C_3$-glyphosate and 49% of $^{13}C_6$-metamitron equivalents) than in the water only (2.4% of $^{13}C_3$-glyphosate and 4.9% of $^{13}C_6$-metamitron equivalents). This finding demonstrates the key role of sediments in pesticides' degradation. Nearly all ^{13}C and ^{15}N-NER from $^{13}C_3{}^{15}N$-glyphosate were biogenic NER, whereas 83% of ^{13}C-NER from $^{13}C_6$-metamitron were bioNER. The presented data provide the first evidence of the utilization of glyphosate as a carbon and nitrogen source and metamitron as carbon source. ^{13}C (and ^{15}N-label) incorporation into amino acids revealed that both pesticides were degraded into two different pathways.

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Environmental Fate of Antimicrobial peptides

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Due to resistance formation towards conventional antibiotics, antimicrobial peptides (AMPs) are becoming increasingly important as a last resort against multi-drug resistant bacteria. The World Health Organization has reclassified some AMPs as critically important for human medicine in 2011. However, many AMPs had been introduced to the market before environmental risk assessment was required, e.g., by the European Medicines Agency (EMA) since 1998. While, polymyxins and bacitracin have been administered as antibiotics and growth promotors in the feedstock industry since the 1960sand were reconsidered for human medicine by EMA in 2013, their mobility and persistence in the environment remains unknown. In this study, we investigate the environmental fate of three commonly used AMPs: bacitracin, daptomycin, and polymyxins B and E (Colistin). We find moderate affinity of anionic daptomycin to sorb to standard European soils (K_d=20.6 – 48.6), while the cationic polymyxins bind irreversibly. Assuming moderate mobility for some AMPs and potential to be flushed out by run off events, we further investiated photochemical and microbial transformation processes in surface waters. We demonstrate that daptomycin has a photochemical half life of 7 hours, predominantly driven by direct phototransformation (48%), reaction with singlet oxygen (19%), and with excited triplet sensitizers (up to 33%). Polymyxins have shorter half-lives (1.5-2.0 h) predominantly driven by indirect photochemical processes. Ongoing test further suggest that biotransformation by riverine biofilm can play a key role in the fate of AMPs. Our findings of sorption behavior, photo- and biotransformation rates together will reveal the overall persistence of AMPs in the aquatic environment. Keywords: Antimicrobial peptides, photodegradation, biotransformation

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Natural Bromophenolic Compounds in Nordic Macroalgae

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Macroalgae are used worldwide for human food. They are a rich source of vitamins, minerals, fibre, phytochemical antioxidants (fucoxanthin, polyphenols) and omega-3s, and many health benefits are attributed to their consumption [1]. Bromophenolic compounds (BPCs) are commonly found in marine algae. BPCs comprise bromophenols (BPs) and transformation products bromoanisoles (BAs), hydroxylated and methoxylated bromodiphenyl ethers (OH-BDEs, MeO-BDEs) and polybrominated dibenzo-*p*-dioxins (PBDDs). BPCs enter the human diet through macroalgae consumption and from BPCs bioaccumulated in seafood. Profiles of MeO-BDE congeners in human serum reflect dietary exposure [2]. Toxic properties associated with BPCs include hormone disruption (OH-BDEs and MeO-BDEs) [3] and inhibition of oxidative phosphorylation (OH-BDEs) [4], and binding to the aryl hydrocarbon receptor (PBDDs) [5]. BPCs are accumulated in the Baltic food web [6,7]. In 2017-2018, we collected macroalgae from the Bothnian Sea (northern Baltic), Swedish west coast (Skagerrak) and Norwegian coast to determine the variability of BPCs among species and locations and estimate the role of macroalgae in supplying BPCs to Nordic estuaries. Compounds identified in 18 species (red, green and brown) were 2,4-DiBA, 2,4,6-TriBA, 2'-MeO-BDE66, 6-MeO-BDE47, one tetrabromo- and two tribromo-MeO-BDEs with unknown substitutions. Concentrations (pg g^{-1} wet wt.) varied over several orders of magnitude: 62–41000 for Σ_2BAs and < 15–490 for Σ_5MeO-BDEs. Higher concentrations were generally found in brown algae *Ascophyllum nodosum* and *Fucus spp.* The wide range of BPCs in macroalgae and strong seasonal variations [6] are important factors in controlling sources to estuaries and bioaccumulation, and for human consumption. [1] Brownlee, I. et al. 2012. The potential health benefits of seaweed and seaweed extract. In: Pomin, V.H. (ed.) *Seaweed: ecology, nutrient composition and medicinal uses. Marine Biology: Earth Sciences in the 21st Century*. Nova Science Publishers, Hauppauge, NY, 119-136. [2] Haraguchi, K. et al. 2016. *Environ. Internat. 97*, 155-162. [3] Wiseman, S.B. et al. 2011. *Mar. Pollut.Bull. 63*, 179-188. [4] Legradi, J. et al. 2014. *Environ. Sci. Technol. 48*, 14703-14711. [5] van den Berg, M.; et al. 2013. *Toxicol. Sci. 133*, 197-208. [6] Dahlgren, E. et al. 2016. *Chemosphere 144*, 1597-1604. [7] Dahlberg, A.K.M.; Chen, V.L.; Larsson, K.; Bergman, Å.; Asplund, L. \n2016. *Chemosphere 144*, 1475-1483.

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Poster spotlight

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Remediation of Contaminated Environments and Innovative Methods in Biological Strategies (II)

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Biodegradation of cylindrospermopsin by manganese oxidizing bacteria
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Cyanobacterial blooms pose a high risk to the health of humans and freshwater organisms due to the potential production of cyanotoxins. Cylindrospermopsin is an alkaloid toxin commonly found in freshwater bodies and is toxic to humans and other organisms. In natural environments, concentrations have been found between 0.2 to more than 200 $\mu g\ L^{-1}$. So far, little is known about the biological degradation of cylindrospermopsin. Manganese oxidizing bacteria (MOB) are capable of transforming a variety of organic and inorganic pollutants. However, their potential for biodegradation of cyanotoxins, as cylindrospermopsin, has not been assessed yet. Therefore, cylindrospermopsin degradation via different neutrophilic aerophilic MOB isolated from natural and technical systems was investigated. Tested MOB's included one *Pseudomonas* sp. strain, two *Leptothrix* sp. strains, and one *Ideonella* sp. strain. In addition, we assessed the influence of different parameters such as the presence of iron, yeast extract and manganese on the degradation of cylindrospermopsin. The concentration of oxidized manganese, as well as cylindrospermopsin removal by biogenic oxides alone was also evaluated. *Pseudomonas* sp. strain OF001, degraded about 100% of the toxin in three days. The highest degradation efficiency was measured when $MnCO_3$ was used as manganese source for the MOB in the media, ranging from 25 to ~100% of cylindrospermopsin removal and degradations rates of 1.13 – 37.01 $\mu g\ L^{-1}\ day^{-1}$. In the absence of manganese, the degradation was null or lower than 20%. The concentration of oxidized manganese in the medium did not influence cylindrospermopsin degradation, showing that the mere active oxidation of manganese is important without considering how much biogenic oxides they produce. Cylindrospermopsin degradation by biogenic oxides, in the absence of viable cells, was low or non-detectable. Considering the efficient removal of cylindrospermopsin by the tested strains, manganese oxidizing organisms could play an important role in cylindrospermopsin degradation. In addition, MOB could be promising candidates for future enforcements for biodegradation of cylindrospermopsin in water treatment plants.

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Humic products for soil remediation technologies: chemical properties and biological activity
O. Yakimenko, Moscow State University / Soil Science Faculty
Humic products (HP) produced by companies from various organic materials, are being increasingly used in soil remediation. Depending on organic source material (lignite, peat or composts), humic acids (HA), which are considered to be the active ingredient in HP, are different in chemical structure and in their efficiency as plant growth promotors and soil conditioners. The objective of the study was to evaluate in a number of laboratory, pot and field experiments the potential of HP as plant growth stimulators for agriculture and as remediating agents for contaminated soils. Chemical properties of a number of lignite-, peat- and lignosulphonate- derived HP (elemental content, IR-spectra, absorption and fluorescence spectra) are analysed. Biological activity of the same HP are examined in laboratory and field experiments using various endpoints: plant biometry, soil chemical and biochemical parameters, bioassays with battery of test-cultures, effect on yield and crop quality in field trials. Potential of HP to decrease content of available species of heavy metals in contaminated soils is evaluated in case studies. Several approaches to assess the effectivity and "integrated environmental quality" of HP are tested and compared using detoxication coefficient, Triad method, and Harrington desirability function. The organic matter genesis is the key factor influencing composition and structure of HA in HP. HA from fossil coal are more aromatic, hydrophobic, contain fewer functional groups and mobile low-molecular components. Peat-derived HA are the most aliphatic, hydrophilic and enriched with amino acids and carbohydrates. Using absorption and fluorescence spectra one can discriminate organic matter source for HP from lignite, peat and lignosulphonate. Bioassays showed that HP could provide both stimulationg and inhibiting effect, depending on sensitivity of test-culture, experimental design, nature and properties of HP. In laboratory tests most of analyzed HP promoted development of roots and shoots, stimulated fluorescence of algae and affected activity of some soil enzymes at 10-100 mg/L. In field trials, preplant seed treatment with HP affected plant resistance to chemical products. In heavy metal contaminated soils application of HP decreased the toxic effects of Cu, Pb and Cd on plants. Integrated "environmental quality indicators" showed that HP at rates 0.0025 and 0.01% are able to reduce ecological toxicity in urban soils The study was supported by RFBR, grant 18-04-01218_?

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Antioxidant activity and toxic properties of humic substances. Evaluation via bioluminescent enzymatic assay
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Bioluminescent methods are widely used to monitor toxicity of media due to high rates of the assay procedure and simple registration of the luminescent signal. These features provide a large number of measurements under comparable conditions, which is extremely important for statistical processing. The classic bioluminescent bioassay is based on luminous marine bacteria; it has been widely used for more than 50 years to assess a toxicity of aquatic environments due to high sensitivity to pollutants. A suppression of maximal bioluminescence intensity is a quantitative characteristic of general (integral) toxicity of the environment. Recent decades, a new type of bacterial bioluminescence assay develops. It is based on a system of coupled enzymatic reactions of luminous bacteria, namely, two reactions catalyzed by bacterial luciferase and NAD(P)H:FMN-oxidoreductase. A particular feature of this bioassay is specificity to the group of toxic agents of oxidative nature: additionally to reducing maximum luminescence intensity, a bioluminescence delay period occurs; it depends non-additively on the oxidizer' concentration and redox potential. This feature is a basis for monitoring the oxidative toxicity of the environment. Hence, the bioluminescent enzymatic bioassay can be used to determine both the General Toxicity (*GT*) of high-concentration solutions of bioactive compounds (involving humic substances, *HS*) and their ability to change Oxidative Toxicity (*OxT*) of the media, i.e. their antioxidant activity. Toxicity and antioxidant activity of *HS* was studied in a wide range of their concentrations using the enzymatic bioluminescent bioassay. Chemical and biological aspects of the effects are under consideration. The antioxidant activity was demonstrated at low *HS* concentrations. Toxicity of the *HS* solutions was evaluated with effective concentrations *EC-50*; detoxification coefficients D_{OxT} and D_{GT} were used to evaluate antioxidant activity in solutions of organic and inorganic oxidizers. The toxicity and antioxidant activity of *HS* was compared to those of the other bioactive compound, fullerenol-60 (*F*). Differences in the effects of *HS* and *F* were related to (1) the mobility of functional groups and fragments of *HS*, (2) their high ability to reduce the content of reactive oxygen species in solutions. \n The work was supported by the PRAN-32, grant of RFBR N18-29-19003mk

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Structure and dynamics of microfungal communities in soils with different humus content under polymetallic pollution
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Heavy metals (HM) pollution is a priority negative impact on soils. The sorption of HM cations by soils, and consequently the toxicity of these pollutants, depends on soil properties including humus status. Structural indicators of fungal

communities and proportion of melanin containing species can be considered as the most informative for certain kinds of anthropogenic impacts. The humus status of soils can be modeled by the introduction of carbon containing compounds. The aim of the study was to characterize the structure and dynamics of fungal communities under polymetallic pollution in soils with different humus content without and with carbonaceous amendments. The two types of soils were taken for the experiment: chernozem and agrozem (organic carbon content 5.5±0.02% and 1.5±0.02%, respectively). Dry mixture of HM (Zn, Cu and Pb) was added to soil samples. Then the amendments were applied: 5 % biochar in dry form, 0.25 % lignohumate in the form of an aqueous solution, and their mixture. Thus, 8 experimental variants were formed for each type of soil. The inoculation of microfungi from each experimental variant was carried out on three nutrient medium. Accounting of colony forming units (CFU) and taxonomic identification of spore forming forms was carried out. Also the assessment of microbial community structure differences in polluted and amendment soils samples were conducted by means of lipid analysis. At the beginning of the experiment, only minor fluctuations in fungal species diversity, which were little dependent on the type of soil, were revealed. Microfungal communities have undergone a transformation during the exposition. The dynamics of the taxonomic composition and the number of CFU differed in soils of different humus status. We can assume that at the end of the exposure: 1) species diversity of microfungi increased in the both types of soils, but it was higher in agrozem samples; 2) polymetallic pollution had a stressful effect, resulting in an increase in the number of species in the most experimental variants, especially in chernozem samples. According to results of lipid analysis the fungal biomass of dark-colored *Aspergillus* sp. increased sharply under HM in agrozem, and it decreased when the samples were carbon enriched after treatment with biochar and lignohumate. On the contrary, in humus-rich soil - chernozem, the effect of both TM and amendments was not so noticeable. The study was supported by RFBR, grant 18-04-01218_?.

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Electrophoresis of soil humic acids - the key tool for revealing of their structural organization
O. Trubetskoj, Russian Academy of Sciences; O. Trubetskaya, Institut of Bioorganic Chemistry RAS
Electrophoresis of soil humic acids – the key tool for revealing of their structural organization Oleg Trubetskoj[a], Olga Trubetskaya[b] [a]Institute of Basic Biological Problems, RAS, 142290, Pushchino, Moscow region, Russia [b]Branch of Institute of Bioorganic Chemistry, RAS, 142290, Pushchino, Moscow region, Russia E-mail: olegi03@yahoo.com **1. Introduction** One of the electrophoretic approaches, which have been successfully used for humic acids (HAs) study, is polyacrylamide gel electrophoresis (PAGE) where distinct electrophoretic zones (EZ) were observed. On the other hand by using practically all known analytical techniques a huge number of individual molecular structural components were detected in HAs and they are unlikely could represent only a few distinct EZ of soil HA. On this reason the question concerning to EZ of soil HA is - what they are present itself? **2. Objectives** Twelve soil HAs of different genesis were the objectives of present study. **3. Materials & methods** Trubetskoj et al. (1991) has developed the new method of PAGE in the presence of denaturating agents which allowed separation of soil HA into quite reproducible distinct EZ. The coupling PAGE with SEC allowed to obtain preparative quantities of EZ from different soil HAs (Trubetskoj et al. 1999). Only after application of coupling SEC-PAGE for fractionation of soil HA the study of physical–chemical properties of their EZ was possible. **4. Results** We followed systematically this method over the last twenty years with different analytical tools and this allowed us to obtain physical-chemical characteristics EZ of different soil HAs with clear differences in terms of (1) electrophoretic mobility, (2) molecular size, (3) fluorescence and UV-vis spectra, (4) humic-like and protein-like fluorophores distributions, (5) amino acids weight content, (6) plant and/or microbial long chain fatty acids content, (7) aromatic/aliphatic components ratio, (8) hydrophobicity, (9-10) photosensitizing and phytohormone-like activities. **5. Conclusion** EZ of soil HAs independently on the soil origin consist of predominantly aromatic or aliphatic structures and possess different physical–chemical properties. It seems similar marked fractions have similar structural organization. Findings are consistent with the hypothesis that the structure of soil HAs arises through supramolecular assembly of different compound. **Acknowledgements** The work has been supported by RFBR (project 18-016-00078?).

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Selection of amendments to decrease the mobility and toxicity of inorganic pollutants (Pb, As, Ba, Zn) in a mine tailing for the development of a phytoremediation process
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In mining sites, the alteration of tailings induces a risk of dispersion of toxic elements, in addition with potential pollution of groundwater and surface water through leaching. The development of a vegetal cover is one of the potential options to decrease the risks. However, the growth of plants on mine tailings is generally hampered by several parameters: acidity, lack of nutrients, poor texture and toxicity of metals and metalloids. Thus, amendments are necessary to overcome these limitations. A mine tailing material was sampled on a former silver and lead mining site. Chemical analysis indicated the following concentrations in pollutants: 26 432 mg.kg^{-1} Pb, 265 mg.kg^{-1} Zn, 1 063 mg.kg^{-1} Ba, 1134 mg.kg^{-1} As. The tailing is acidic, siliceous and coarse (76% of particles with size in the range of 315 μm - 2 mm). A cow manure and some ochre (iron oxide-hydroxide) material produced in the water treatment plant of a coal mine were tested, alone or in combination, to stabilize the most mobile toxic pollutant, Pb, while avoiding the mobilization of other toxic elements. Leaching and microcosm experiments were performed and showed that the Pb concentration in water, that was higher than 10 mg.L^{-1} with the not-amended tailing, was decreased by a factor ×100, while As, Zn and Ba concentration did not increase significantly. Ochre (added at 5% in weight/weight) allowed increasing pH and could adsorb some metals and metalloïds. The metabolic microbial diversity, evaluated using Ecolog® plates, was improved by all amendements after one month of incubation. Polymers were the most used substrates in the non-planted microcosms, after one month of incubation, suggesting a specialized microbial community. Planted microcosms revealed that the growth of ryegrass was significantly improved by the addition of ochre and manure : the biomass of ryegrass is 3.5 X (with 5% ochre and 2% manure) to 4.8 X (with 5% ochre and 0.15%) greater than in the condition of plants growing on unamended soil. The next experimental step will include the monitoring of phytostabilization at metric scale, in mesocosm, with a vegetal specie naturally present on Pontgibaud mine site, belonging to the *Agrostis* genus.

From Hazard Assessment to Regulatory Risk Management Action - Approach Development for UVCBs (II)

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Approaches to address variability and uncertainty in PBT and risk assessment of Petroleum substances
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1. Introduction Risk assessment of petroleum substances is complicated by the variable and complex nature of the composition of the substances. Analytical characterization methods used to characterize composition vary based on project needs as well as the nature of the substance. The analytical techniques for complex substances often are challenged to definitively identify every constituent in a substance, which introduces some uncertainty into the risk assessment process. Environmental fate and toxicity properties also vary by chemical class and carbon number and is characterized by using representative structures with known, or estimated, physicochemical properties. Risk assessment frameworks need to be flexible to account for these variable analytical inputs and compositional variability. Existing risk assessment frameworks use the hydrocarbon block method to reduce variability and uncertainty. Variabilty is assessed by applying risk assessment models across broad datasets to understand the extent of variation between and within categories. Uncertainty in risk assessments is accounted for by comparing toxicity and risk simulations using estimated structures, compared to empirical toxicity data, and conservatively derived predicted no effect thresholds (PNEC). Further, uncertainty is further addressed through the use of conservative emission factors that can greatly over-estimate true emissions. 2. Materials and methods The hydrocarbon block method used for several decades and recent applications rely on finer blocks corresponding to avaible analytical chemistry in the PETRORISK model [1], and has been used in the PETROTOX model for toxicity prediction of petroleum substances [2, 3]. Both models use the toxicity thresholds established in the Target Lipid Model [4]. Application of these models to a wide range of petroleum substances provides an indication of the variability that is observed between substance categories, sources, seasons, and other factors that can affect substance composition. Uncertainty in PBT assessment was evaluted by comparing model predictions to available acute and chronic toxicity data for petroleum substances and through comparison to the PNECs [3]. 3. Results and discussion Variability exists in all model frameworks as a general rule. The impact of the variability on toxicity predictions for risk assessment is minimized by using statistical extrapolation techniques to estimate conservative PNECs. The PNECs are validated against available chronic toxicity data. 4. Conclusions In case of PBT assessment we advocate that uncertainty should be addressed using weight of evidence evaluation using multiple lines of evidence, and especially higher tier evaluation data. This would improve transparency and predictability in the decision making process. Otherwise, the inconsistent use of data and lack of WoE will effectively mis-prioritize chemicals limits the ability of industry to generate reliable data to support sound risk assessments. 5. References Redman, A.D., et al., *PETRORISK: A risk assessment framework for petroleum substances.* Integrated environmental assessment and management, 2014. **10**(3): p. 437-448. 2. Redman, A.D., et al., *PETROTOX: An aquatic toxicity model for petroleum*

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A proposed Method of Determining Environmental Risk of Complex Substances
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1. Introduction Methodologies for Environmental Risk Assessment (ERA) for single substances have been well established in the EU for some time. However, these assessments are not designed to be perfect, tend to conservatism, include non-science-based assessment factors to account for uncertainty and cannot depict with precision the real fate of a chemical. Current ERA methodology uses assumptions and approximations, e.g. extrapolation of hazard and risk to the sediment compartment from the pelagic compartment. Because the studies of toxicity to sediment-organisms are difficult to perform and are only required for higher tonnage bands, the concept of equilibrium partitioning (EP) is used and this relies on the hypothesis that the toxic effect concentration attributed to the most sensitive aquatic invertebrates can also be applied to benthic and terrestrial species as the pore water concentration. Despite this, the EU ERA method for chemical substances is considered as a useful tool by regulatory ecotoxicologists and substances with a RCR less than 1, are generally deemed of low concern for the environment. However, the EU risk assessment methodology is designed only for mono-constituents while many chemical substances falling under REACH regulation are multi-constituents or substances of UVCBs. Should every constituent be taken into account, and if so, is there a point at which their influence is limited? Is there a way in which the mixture is treated as a whole without overloading dossiers with multiple risk assessments? Aquatic toxicity of mixtures can be experimentally assessed using water-accommodated fractions (WAF) for partially soluble multi-constituent substances. In a WAF study the different constituents of the mixture are tested together at several loading rates. Toxicity is expressed as a single value of loading rate for the whole mixture. However, a toxicity result derived from a WAF study cannot be directly transposed to sediment or soil hazard and risk limiting the possibilities of performing an ERA for other compartments than the aquatic toxicity by the EP method as is traditionally carried out for single substances. KREATiS recently developed a calculation method for WAFs which has been supported by numerous experimental studies and which provides a mechanistic explanation for the WAF results using a thermodynamic approach. In this presentation, we consider moving a step further using a complementary blend of the WAF calculation and the Di Toro method in order to calculate Predicted Environmental Concentration (PEC) and Predicted No Effect Concentration (PNEC) values that have the same units (chemical activities) and allow the calculation of RCRs for mixtures in all environmental compartments. 2. Methodology The methodology proposed is divided into 3 main steps: hazard (acute and chronic) of the mixture for aquatic organisms and for benthic organisms has to be determined. exposure is determined in the aquatic compartments for each constituent using the standard methodology proposed in the ECHA guidance dedicated to environmental exposure. hazard (as PNEC) and expsoure (PEC) are converted into Predicted No Effect Activity (PNEA) and the Predicted Exposure Activity (PEA) of a mixture in the different compartments to give the RCRs per compartment As an example, an artificial equimolar mixture was formulated using an equimolar mixture of the 4 constituents. The constituents were selected ranging from slight to high hydrophobicity to achieve a relatively heterogenous distribution between water and sediments (such differences are typically observed in essential oils). Acute and chronic toxicity values to *Daphnia magna* and *Lumbriculus variegatus* have been measured according the OECD guidelines 202, 211 and 225. 3. Results and discussion Experimental tests and calculations were performed to determine the hazad of the mixture. The results showed that the toxicity (acute and chronic) obtained with WAF assays is predictable using a calculation method. Moreover, they showed that extrapolation from pelagic to benthic organisms (as suggested by Di Toro *et al.*) is still relevant for mixtures. 4. Conclusion The relevant ERA for mixtures is feasible by combining an *in silico* generated whole-substance approach (based on individual constituents) to determine the toxicity for pelagic organisms with the EP method to extrapolate effects on benthic organisms. The exposure is determined in each compartment using the EP method per constituent. Finally an activity-based approach is proposed to normalise the relationship between the toxicity and exposure by each constituent in order to determine a homogenous RCR for mixtures in the aquatic compartment using the same units.

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UVCB fate-directed toxicity testing and risk assessment (UVCB-FATETOX)
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UVCBs are substances of unknown or variable composition, complex reaction products or biological materials. UVCB substances consist of many different chemical constituents, some which may be unknown. Examples include petroleum substances, chlorinated paraffin's (CPs), flavoring agents, essential oils & their derivatives, natural oils and extractives, and biofuels. Assessing the environmental and human risks posed by UVCB substances is a challenge currently confronting chemical industry and regulators. This research project focuses at the toxicity and bioaccumulation testing of those UVCB constituents that are persistent. The objectives are (1) to develop approaches for fate-directed ecotoxicity assessment of UVCBs based on new analytical methods, dosing methods, fate directed fractionation, toxicity testing and models, (2) to conduct a case study on selected UVCBs and develop a generic risk assessment strategy for UVCBs and (3) to cross fertilize and partially align ongoing research activities at three European research institutes. The first work package focuses at combining state of the art dosing & analytical techniques with UVCB fate testing and includes (a) Passive dosing by polymer water partitioning & application to toxicity testing, (b) Aquatic biodegradation testing at environmental low concentrations coupled to advanced analytical instruments and (c) Bioaccumulation testing of UVCB mixtures coupled to Headspace-SPME and purge-and-trap. The second work package focuses at fate directed ecotoxicity and bioacumulation testing and includes (d) A literature review on fate-driven toxicity testing, (e) Aquatic toxicity testing with and without fate directed pre-treatment, (f) Bioaccumulation testing linked to biodegradation pre-treatment and (g) Linking toxicity to equilibrium concentrations in polymer and lipid – baseline versus excess toxicity.

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Risk management of inorganic UVCB substances in the metal sector
F. Iaccino, Arche consulting; F. Verdonck, ARCHE; V. VEROUGSTRAETE, Eurometaux / EHS
A typical metal's lifecycle is made of a number of extraction and refinement processes, along which metals can be found in 'mixture' with other metals and mineral constituents embedded in a matrix or in a crystalline structure (e.g. of a pigment), as part of articles used for recovery or constituents of e.g. slags at the end of the refining process. These metal-containing materials represent significant volumes in the European market and an accurate risk assessment is essential to ensure that any potential environmental and health risk associated with their production, transport, storage and use is properly managed. Under the REACH Regulation, these substances are identified as UVCBs or substances of Unknown or Variable composition, Complex reaction products or Biological materials. Typically, their uncertaintyis linked to the analytical identification of some constituents' speciation (despite the elements in the composition are known and can be accurately quantified), whereas the variability is revealed by the broad concentration ranges for various constituents. Since 2013, Eurometaux is working on the development and implementation of an appropriatemethodology for the assessment of the inorganic UVCBs in the metals sector, i.e. from substance identification to risk assessment and risk management measures (RMMs) designation. The management of the risks posed by these materials, which is the ultimate goal of the REACH assessment, necessitates the identification of any possible hazard and assessment of exposure; however, testing eco/toxicity on the substance itself encounters several limitations considering the variability in the composition and the difficulty in identifying a set of representative and conservative samples. A constituents-based approach was therefore developed. In a nutshell, human health and environmental toxicity is considered to be driven by the toxicity of the UVCB constituents. To account for the uncertainty and variability, a precautionary approach is used along the different stages of the risk assessment by assessing reasonable worst-case speciation concentration. For the hazard classification, rules defined for the hazard assessment of mixtures under the UN Globally Harmonised System (GHS) and the EU Classification Labelling and Packaging (CLP) are applied. The MeClas (www.meclas.eu) web-based tool is used to ensure transparency in data selection, processing and reporting, compliant with GHS and CLP requirements. It used for hazard identification and classification of complex inorganic materials like UVCBs or others, based on the hazard of their constituents and on higher tier refinements, when e.g. bioavailability correction are available. Hazard classification for local effects leads to specific risk management measures. Similarly, the effect assessment for systemic and environmental effects is based on the specific assessment of the UVCB individual hazardous constituents, for which the risk assessment is conducted separately (and REACH registrations already exist) and on their (possible) combined effect. The exposure, risk assessment and hence selection of risk management measures is often based on representative measured data of their constituents, which are integrative of exposure to multiple UVCB substances at an industrial site. This reduces the level of uncertainty in the assessment and captures the variability in the composition. Common risk management strategies for the environment are for emissions to air: (wet) electrostatic precipitators or cyclones or fabric/bag filter or ceramic/metal mesh filter; for emissions to water: chemical precipitation or sedimentation or filtration or electrolysis or inverse osmosis or ion exchange. Emission to waste are low given the high internal or external metal recovery/recycling in the sector. Common risk management measures for the occupational exposure are use of fully or partially closed systems, integrated or

exterior local exhaust ventilation and capture sprays or wet suppression techniques.

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Panel discussion

Panel composed of Eric Verbruggen (RIVM, Netherlands), Paul Thomas (Kreatis, France), Philipp Mayer (DTU, Denmark). Interactive discussion on the learnings and regulatory implementation Summary/conclusions from what has been presented + set of questions for the panel Wrap up

Micro(Nano)plastic Pollution: Tackling the Plastic Problem by Identifying Sources, Investigating Fate and Novel Approaches (III)

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New sampling strategies for optimised microplastic analysis in surface waters
U. Braun, BAM- Federal Institute Materials Research and Testing / 5.3 Mechanics of Polymers; K. Altmann, P. Eisentraut, Bundesanstalt für Materialforschung und -prüfung; A. Barthel, C. Bannick, Umweltbundesamt; M. Ricking, UBA Umweltbundesamt; M. Reiche, Umwelt-Geräte-Technik GmbH
The occurrence of microplastic particles (MP) in nearly every environmental compartment but especially in water is well documented in an increasing amount of publications released within the last few years. However, a comprehensive understanding of MP occurrence, sources and pathways in the environment as well as effects of MP on the environment are still unclear. Furthermore, the existing methods are often very time consuming, need high user knowledge and labour force, which is often not acceptable for a routine monitoring of an action or legal regulations. The calls for harmonised methods become loud and louder and promising efforts are made in this area. However, the scientific discussion is often focused on advantages and disadvantages of detection tools and sample preparation, less initiative is given to the sampling methods. That is a pity, because every environmental scientist knows, reliable analytical results depend on a good sampling method. At first glance, the collection of samples from aqueous systems seems easy because the sampling process already includes a content of the MP by means of sieves and filters. However, this filtering of water is often limited by the development of filter cake on the sieves, consisting of high amounts of natural particular matter. Hence often a small water volume is filtered, which will not contain a representative amount of trait carriers. Alternatively, complex sieves cascades are used which need complex technical equipment. Recently, we presented a fast thermoanalytical method for the detection of polymer fractions in water samples. This method, thermo-extraction desorption gas chromatography mass spectrometry (TED-GC-MS) can analyse MP in water filtrates without sample preparations within 2-3 hours. Advantageously, rubber particles from tires besides the MP can be analysed simultaneously. In order to become even faster we now start to develop new sampling strategies, which should be usable for different water systems. It is intended to reduce the effort in the field and to elongate times for integrative sampling to obtain representative result without random effects. In the present presentation we will show first result using a sedimentation box for sampling of MP from surface water. Advantages and disadvantages will be discussed and comparison to data derived from conventional fractionated filtration will be given.

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Synthesis of metal doped nanoplastics and microplastic fibers and their utility for investigating plastic fluxes in complex matrices
S. Frehland, R. Kaegi, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering; R. Hufenus, Empa Swiss Federal Laboratories for Materials Science and Technology; D.M. Mitrano, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering
Reports on the occurrence of particulate plastics (nano- and microplastic particles and fibers) in the environment emerge on a weekly basis, but quantitative data are still limited due to analytical difficulties and inconsistencies of the methods applied to detect particulate plastics in complex environmental matrices. While progress is still ongoing to develop analytical methods to measure particulate plastic in field studies, researchers who study the fate, transport and biological interactions and effects of nano- and microplastics in bench top or pilot scale studies can take advantage of an entirely different approach. In this work, we synthesized a variety of particulate plastics (nanoplastic particles, fibers) with an embedded inorganic fingerprint (Pd or In; approx. 0.5% metal/wt) which can be used to detect plastic by common analytical techniques for metals analysis, such as ICP-MS. This allows us to more quickly and quantitatively assess plastic in complex matrices than is currently possible wit h other analytical techniques. To highlight the utility of this approach, here we used these materials to investigate the fate and transport of particulate plastic in a pilot-scale WWTP representing the activated sludge process. Triplicate samples were taken from the mixed liquor and effluent at least twice a week for the entire length of the experiment, which lasted five weeks. With a recovery rate of plastics over 90%, our findings show that over 98% of plastics (both nanoplastic particles and microplastic fibers) were found in the sludge, with a high correlation between TSS concentration and plastic concentration. Therefore, these materials will be removed from the wastewater stream with the sludge. While surveys of municipal treatment plants have shown similar trends in the magnitude of microplastics removed, we can now validate the retention of nanoplastics and microplastic fibers with a more complete mass balance study. With a better understanding of emissions from WWTP, one could suggest estimated annual loads of particulate plastics to surface waters or applied to fields with sewage sludge, which could, by extension, be used as a starting point for fate modelling. Beyond the case study specifically highlighted here, these metal laden particulate plastics are suitable to study fate, transport, eco-toxicity and interactions with organisms at trace concentrations.

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Microplastics in urban stormwaters - designing a method to evaluate microplastic concentrations in stormwater run-offs
J. Talvitie, Finnish Environment Institute SYKE / Marine Research Center
Urban stormwaters have been suggested to act as a significant pathway for microplastics (MPs) to the aquatic environment. However, the actual data of MP concentrations in stormwater run-offs is scarce. In addition, there is no validated methods to sample, extract and analyse MPs from stormwaters. In this study, a flow-based high-volume sampling concept that gives event mean concentrations (EMCs) of MPs for sampled rain events was developed. The sampling concept is based on flow-based pumping and in-situ filtration. The samples are collected from the stormwater stream in the stormwater well. First, the V-dam is installed to the discharge side of the well. This makes the surface level of the stormwater stream in the well proportional to the flow rate of the stream. To determine the flow rate in real time (delay < 10 ms) the surface level is followed by using level transmitter. Programmable Logic Controller (PLC) collects and saves this information and uses it to control electrical pump with frequency converter. Pump is used for creating sample flow proportional to the stormwater flow. Also sample flow is saved to the PLC. This information is then stored to Cloud Server and can be accessed with PC either real time or later. PC programme and phone app is developed to follow and control the process. The sampling is performed repeatedly from one site with well known catchment area. As the quality of the stormwater is known to fluctuate due to several factors (e.g. land type and use, intensity and depth of rainfall, antecedent weather conditions), it is necessery to sample adequate amount of rain events to ensure reliable estimation of the total microplastic discharge. Enzymatic sample purification processes and automated FTIR analyses techniques will be applied with modifications for stormwater samples to gain high quality data.After the sample processing and analyses is performed, the EMC of MPs as particle account as well as in mass will be evaluated. After the sampling period (~ 6 months), the site mean concentration (SMC) of MPs will also be calculated for the catchment area.

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Preparation of small microplastics and nanoplastics: challenges, technical gaps and environmental relevance
G. Balakrishnan Nair, Le Mans Université / IMMM Le Mans Université; M. Revel, Catholic University of the West; C. Mouneyrac, Catholic University of the West / UBL, Mer Molécules Santé; A. Huvet, Ifremer, LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer; I. Paul-Pont, LEMAR UMR 6539 CNRS; P. Soudant, LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer; f. lagarde, Institute of molecules and materials of Le Mans / Institute of Materials and Molecules of Le Mans IMMM UMR CNRS
Model particles of different sizes and nature of polymers are of great interest for improving knowledge on micro and nanoplastics impacts on organisms and helping their physical and chemical characterizations in aquatic samples. However, in the smallest size range (< 10 μm), particles of only a few types of polymers are currently available, especially commercially. For this reason, most toxicity tests were reported using PS micro and nanobeads whereas polyethylene (PE) and polypropylene (PP) fragments are by far the most common in the aquatic environments. In addition, commercial micro and nanobeads are usually stabilized using surfactants without knowledge of the additives formulation, which may modify their interaction pathway with aquatic organisms. So far, only a few studies reported protocols to prepare model micro and nanoplastics and some of them present cryomilling as an effective way to obtain reference microplastic particles. However, in these studies, the so-obtained particles are always in the range of several hundreds of μm and no study focused on the production of particles in the range < 100 μm or at the nano size with a standardised size range. In this work, we investigated the possibility to obtain consistent quantities of small particles of PE and PP by milling. The obtained results show that cryo-milling is a fast method to produce microplastics under powder form of all kinds of polymers with sizes > 50 μm. However, producing consistent quantities of particles < 50 μm or even smaller (nanoplastics) by this way remains challenging. We also present all the steps that are necessary to obtain the full characterization of produced particles. It shows that particles < 50 μm were produced but not easily extracted from the powders, a liquid extraction becoming necessary. It

underlines that after milling, the separation of particles in distinct size classes is also very challenging and the potential presence of particles smaller than expected has to be thoroughly checked. Overall, the necessity of a deep characterization in numbers and size of micro and nanoplastics produced by milling appears here primordial for impact assessment studies on biota. Finally, in this presentation, the environmental relevence of the particles produced by milling will be discussed regarding their composition, size, surface chemistry and stability in solution and species.

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Identification and quantification of microplastic by Raman microspectroscopy
N.P. Ivleva, P.M. Anger, Technical University of Munich / Chemistry Department, Chair of Analytical and Water Chemistry; E. von der Esch, Technical University of Munich / Chemistry Department, Chair of Analytical Chemistry and Water Chemistry; C. Schwaferts, Technical University of Munich / Chemistry Departament, Chair of Analytical Chemistry and Water Chemistry; R. Niessner, Technische Universität München / Chemistry Department, Chair of Analytical and Water Chemistry; M. Elsner, Technical University of Munich / Chemistry Department, Chair of Analytical and Water Chemistry
The contamination of aquatic environment and food with microplastic (synthetic polymer particles in a size range of 1 μm – 5 mm) is of increasing scientific and public concern. However, the degree of contamination remains uncertain – e.g. the values for environmental samples range from less than one to more than million items per cubic meter. The contamination of environmental and food samples with smaller plastic particles (< 1 μm) remains unknown. To date, no appropriate analytical techniques for reliable analysis of submicro- (100 nm – 1 μm) and nanoplastic (< 100 nm) are available. While microplastic with sizes down to 1 μm can be both quantified and identified through morphological and chemical analysis by Raman microspectroscopy, the development of an automated analysis for smaller particles (down to the nano-range) is still outstanding. In this presentation, the feasibility of Raman microspectroscopy for the identification and quantification of (pigmented) microplastic as well as paint particles in environmental and food samples will be discussed. The focus will be on the optimization of the technique for automated analysis of plastic in the lower micro-, submicro- and nano-range, as these particles are of high interest due to their increased bioavailability and, hence, potential threat to aquatic organisms and humans.

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Poster spotlight

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Fate and Effects of Metals: Advances in Metals Risk Assessment and Regulatory Guidance (I)

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Contribution of zinc from household and personal care products to wastewater
O.L. Tran, A. Peters, G. Merrington, wca; R. van Egmond, Unilever; J. Kilgallon, Unilever / Safety and Environmental Assurance Centre SEAC
Zinc is a naturally occurring essential element which is both required to some extent by all organisms, but is toxic if present in excess. It is ubiquitous in the environment and occurs at low concentrations in both drinking water and food, but is also introduced into the environment by anthropogenic activities. Zinc compounds are widely used in home and personal care (HPC) products, such as sunscreens, cosmetics and laundry detergents. These products often leave a household via the drain and end up in the wastewater system. However, there is an increasing academic and regulatory interest in 'down the drain' chemicals in HPC products contributing to environmental effects. Wastewater treatment may range from no wastewater treatment to sophisticated tertiary treatments capable of specific substance removal. Different treatment conditions can affect the fate and transformation of zinc in the effluent, where the speciation of zinc is a crucial determinant of the bioavailability and toxicity to aquatic and terrestrial organisms. This study considered the importance of different sources of zinc into the environment via municipal wastewater systems and the contribution from ambient backgrounds concentrations of zinc in environmental media. The results from a systematic literature review show that HPC products are likely to make a relatively limited contribution to overall loadings of zinc to sewage treatment plants, but a larger proportion of the total effluent zinc concentration in domestic wastewaters. Ambient background levels of zinc in foods result in significant quantities of zinc in faeces, accounting for 60 to 70% of the zinc loading in domestic wastewater and more than 20% of the zinc loading in mixed wastewater from domestic and industrial premises. Wastewater containing HPC products were estimated to make up approximately 15% of the domestic wastewater stream, thus these products are a relatively minor contributor to total zinc loadings in wastewaters. The output of this work may be useful in the environmental risk assessment of zinc for HPC products and may be used to inform regulatory strategies and priorities for zinc.

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Mercury accumulation in Mediterranean lean fish
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Mercury is primarily associated with muscle tissue rather than with fat which, besides oily fish, points to predatory but non-migratory fish species, e.g. lean fish, for the accumulation of this metal. The present study is devoted to determine the concentrations of total mercury and methylmercury in a great variety of lean fish species from the Western Mediterranean Sea and to determine how consumption of these specimens contribute to the total mercury intake in human populations, and more specifically children. 607 commercial seafood samples from the Western Mediterranean Sea were collected (Feb 2014-Nov 2018). Samples from Egypt and the Atlantic Ocean (Senegal, Mauritania coasts) were also taken for comparison. Fish species were selected considering the most consumed by the population. The fish species were grouped in three trophic levels, those feeding on plankton (first), on small fish and crustaceans (second) and on fish and cephalopods (third). A considerable number of the analyzed fish species exceeded the maximum levels proposed by the European legislation, such as dusky grouper (100% of the examined specimens), common dentex (65%), conger (45%), common sole (38%), hake (26%) and angler (15%), among others. Representation of the Hg concentrations vs. weight of each specimen from the third trophic level showed a significant positive correlation, r = 0.78 ($p < 0.01$). The average THg intake due to fish consumption, 0.61 μg/g ww, involved Hg estimated weekly intakes (EWIs) of 5.7 μg/kg bw for children aged 7-12 years and 4.4 μg/kg bw for adults when referred to infant and general Spanish population. These values were higher than the provisional tolerable weekly intakes (PTWIs) for total Hg intake recommended by FAO/WHO, 4 μg/kg bw [4], 140% and 110%, respectively. In the worst case scenario, the children and adults were exposed to 47 μg/kg bw and 37 μg/kg bw, which exceded largely the established upper limit, 1200% and 930%, respectively. For MeHg, assuming that 90% of total Hg is in this form, the EWIs were 5.2 μg/kg bw and 4.0 μg/kg bw for the medium case and 42.5 μg/kg bw and 33 μg/kg bw for the worst scenario in children and adults, respectively. The PTWI for MeHg were set to 1.3 μg/kg bw which was lower than the EWIs for children and adults. Accordingly, the observed EWIs for children and adults were 400% and 310% of the PTWIs for MB and 3300% and 2600% for UB respectively. These values represented scenarios in which only local fish was consumed.

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Black pine (Pinus nigra) bark as biomonitors of airborne mercury: sampling and analytical suggestions for minimising methodological biases
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Tree barks are, in principle, excellent adsorbents of airborne pollutants, including toxic metals. Despite this, the use of tree barks for environmental monitoring was not widespread, although growing for air pollution biomonitoring studies. The skepticism of some researchers is based on: a) the need for a deeper knowledge into mechanisms of bark interaction with air pollutants, and b) limited data regarding variability in bark pollutants, both at a single site or between sites, which complicates the assessment of the actual environmental pressure on a given area. Concerning b), there is obviously a need for a commonly accepted sampling procedure to make the results more comparable and, hopefully, more reliable. The present study aims to outline some factors that may minimize sampling biases in the analysis of Hg in barks. In this work, barks of Black Pine *(Pinus nigra* J.F. Arnold) trees from the Abbadia San Salvatore district (Mt. Amiata region, Southern Tuscany, Italy) and the underlying soils were sampled in summer 2016. In this area two industrial activities, past Hg mining and ongoing geothermal energy production, affect local atmospheric Hg levels. For each site, we collected one sample of soil and eight bark specimens from a single tree. Barks were collected at two different heights, 70 and 150 cm, and in the four cardinal directions for each height. Total Hg in barks varies between 0.1 and 28.8 mg/kg dw. Almost systematically, the correlation between sample height and cardinal direction is better for samples picked up at a height of 150 cm with respect to those at 70 cm. A selected set of bark samples were subjected to a preliminary leaching test to evaluate the amount of Hg that could be removed by a rainy event. The results of the test show that the amount of Hg leached is negligible (maximum Hg concentration in the leach solution ~ 0.1 μg/L). For *Pinus nigra* species in the Mt. Amiata region, measured total Hg concentrations are independent of wind direction. To harmonize results on the employment of tree barks as a biomonitoring substratum, we suggest that a convenient sampling practice for Pinus nigra is to collect a 1-2 mm bark slice within the outermost 1.5 cm, at 150 cm from the ground even after a rainy event. Reanalysis of bark after two years showed a decrease in total Hg ranging from 5-20% of original values. While Hg

loss from bark sampels was not extreme, data suggests that barks should be analyzed soon after collection.

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Effects of a sublethal metal mixture of 10%LC50 Cu, Zn and Cd on metal accumulation and ionoregulation in Cyprinus carpio
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The aquatic environment is the final sink of waste streams and receives a huge number of different compounds including metals that can harm the health of aquatic organisms. Considering that we can not predict the toxicity of a mixture on the basis of single compounds, the analysis of mixture toxicity is needed to understand the impact of pollution in an aquatic ecosystem. Even if there are several studies describing the adverse effects of waterborne metals, it is more difficult to find information on the effects of trinomial mixtures. Therefore the main question of the present study is to assess if a mixture of copper, zinc and cadmium at 10% of their respective LC50 can be considered as safe. In order to answer this question, common carp were exposed to Cu: 4.8 μg/L; Cd: 2.9 μg/L and Zn: 206.8 μg/L at 20°C for a period of one week. We looked at fish survival rate and bioaccumulation, with additional physiological parameters to determine if there is an effect on ionoregulation and defensive mechanisms such as MT induction. Our results show a rapid increase in copper and cadmium concentration in gills and liver after one day of exposure. The accumulation of these metals showed adverse effect on ion-homeostasis; for example copper led to a sodium drop in the gills, liver and muscles. The decrease in sodium content affected the whole body ionoregulation, while a decrease in potassium levels was observed in in the liver. An increase in the gene expression for H^+-ATPase and a decreased expression of the Na^+-channel were observed after one day of exposure. We hypothesise that this increase in H^+-ATPase and the subsequent recovery of the Na-channel expression rates are an attempt to compensate for the reduced Na^+ uptake due to competition between copper and sodium at the uptake site. In addition a strong stimulation of metallothionein gene expression was reported during the whole experiment.

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Evaluation of four first tier methods to measure metal(loid) bioaccessibility in polluted soils
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In the French context of polluted site management, the human health risk assessment is usually based on the total metal(loid) concentrations in the soil. Considering that the whole amount of a contaminant is available for organism after ingestion of soil particles, health risks are often overestimated. Moreover, this consideration opposes the concepts of bioavailability and bioaccessibility. To refine exposure and risk assessment, it is recommended to use bioaccessible concentrations of metal(loid)s. In France, the Unified Bioaccessibility Method (UBM) is considered as the oral bioaccessibility reference method. However, for French PSS (Polluted Sites and Soils) managers, this method appears as complex and time consuming, implies high technical skills and needs numerous chemical and biological reagents. Thus, a simplification to measure bioaccessibility of metal(loid)s might be of great interest. The objective of the present study is to propose a first tier method as a decision-making tool for managing polluted sites. The suitability of chemical extractions using acetic acid (0.11 M), citric acid (0.11 M), EDTA (0.16 M) and hydrochloric acid (0.65%) was evaluated to mimic the bioaccessible fraction of As, Cd and Pb in 209 soil samples including all major French soil types and a wide range of physicochemical parameters and metal(loid)s' concentrations. Statistical analyses (i.e. linear correlations) were established to determine the most relevant single-extraction method(s) capable of solubilizing As, Cd and Pb in the same concentration as those measured in both gastric and gastrointestinal phases defined by UBM. Significant relationships were obtained between As, Cd and Pb extracted in gastric and gastrointestinal phases and using the single-extraction methods. The results showed that the dilute hydrochloric acid test could be used as a suitable proxy to assess the gastric bioaccessibility of As, Cd and Pb in a first tier screening (ease-of-use, fast and reproducible method; reduction of analytical costs). Note that if these predicted bioaccessible values indicate that it may be worth to consider bioaccessility in terms of human exposure, then, in a second tier study, the UBM protocol may be used.

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Assessment of metal(loid) bioavailability for landsnails and human bioaccessibility: a new pathway to build bridges between ecotoxicological and human health risk assessment of contaminated soils
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In the current context of polluted sites and soils, human health risk assessment (HHRA) and ecotoxicological risk assessment (ERA) are conducted independently and mainly based on the measurement of total contaminant concentrations in soils. Considering that all contaminants in the soil are mobile and available for organisms, these evaluations are in opposition with the bioavailability concept where only a fraction of the contaminant is assimilated by an organism and consequently cause adverse effects. To estimate more accurately the exposure of organisms to metal(loid)s, some tools exist for human such as oral bioaccessibility test or for the soil fauna such as bioavailability test for snails; however until now they have never been combined to look for possible bridges between HHRA and ERA. Thirty soils contaminated by arsenic, cadmium and lead were selected to cover a wide range of physico-chemical parameters and soil contamination levels. An *in vitro* chemical test of bioaccessibility mimicking the digestion of metallic elements (ME) (Unified Barge Method or UBM) and an *ex situ* biological test assessing the bioavailability of ME to snails were performed. The results of both methods were analyzed using simple and multiple linear regressions and were confronted to look for relationships between ERA and HHRA. For ERA, risk coefficients (RC) associated with bioavailability for snails were estimated based on accumulated contaminant concentrations and their inherent toxicity whereas for the HHRA, hazard quotients (QD) based on contaminant concentration in soils and weighted by the bioaccessibility results were calculated. Results showed an influence of soil parameters on ME bioaccumulation by the snail, *Cantareus aspersus* but not on the bioaccessibility, possibly due to the high acidic pH in the UBM test (compared to the neutral pH of the snail digestive tract) that can cause significant desorption of contaminants and limits the impact of soil parameters such as organic matter. Relationships established between bioaccumulation and bioaccessibility revealed adjusted R^2 between 0.73 and 0.94. In addition, correlations were highlighted by comparing RC and QD. For the three ME studied, a strong relationship exists between the ERA and HHRA methodologies. These results provide a basis for future ecotoxicological and human health risk assessment approaches of polluted sites and soils for organisms with various physiology and way of exposure.

Antimicrobial Resistance in the Environment

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Contamination of antibiotic resistance genes in the Pearl River watershed and representative anthropogenic sources
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Antibiotic resistance is a global public health concern. China is the world's largest producer and consumer of antibiotics. A large number of antibiotics or antibiotic resistant bacteria/genes (ARGs/ARB) are discharged into rivers via domestic, hospital, and industrial wastewater. Urban rivers are considered as the sources and sinks of ARGs/ARB discharged by human activities. The distribution pattern, environmental pollution load and potential anthropogenic sources of antibiotics and ARGs in urban receiving water are needed to be explored. To investigate the impact of urban activities on the spread of antibiotic resistant, this study investigated the pollution characteristics of ARGs in the Guangzhou section of the Pearl River watershed. The removal rates of ARGs and antibiotics from typical anthropogenic sources by wastewater treatment facilities and the discharge load of ARG and antibiotics into the urban receiving waters were analyzed. Contamination of ARGs were determined by quantitative PCR. Concentrations of antibiotics were analysed for the collected samples following our previous method. Results showed that antibiotics and resistant genes were introduced into the receiving river directly or indirectly by representative anthropogenic sources via wastewater discharge. Landfills and livestock farms can effectively remove antibiotics and resistant genes. Municipal sewage treatment plants, pharmaceutical factories and hospitals should enhance their removal efficiency of antibiotic and ARGs from their wastewater. The total amount of antibiotics and ARGs discharged into the pearl river by the municipal sewage plant was 1,450 kg/year and 1.2E+21 copies/year, respectively. The pollution of ARGs and antibiotics in the Guangzhou section of the Pearl River watershed was closely related to urban anthropogenic activities. This may not only exacerbate the contamination of common environmental genes in waters, but introduce the undetected and clinically relevant ARGs into the urban receiving waters as well.This research has provided indispensable data on the distribution and dissemination of ARGs from various source in urban receiving waters and on the relationships between antibiotics and ARGs. These data will be helpful for risk assessment of ARGs and for government policy making. Future research is warranted to understand the mechanisms for the dissemination of ARGs/ARB in urban receiving waters.

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Transport of antibiotic resistance exceeds their selection by residual antibiotics in wastewater-impacted rivers

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Wastewater treatment plants (WWTPs) are point sources for both, the release of antibiotic resistance genes (ARGs) and the discharge of antibiotics (ABs) into the environment. While it is well established that ARGs emission leads to an ARGs increase in receiving rivers, the role of sub-inhibitory AB concentrations is also being discussed. However, the results obtained in this study suggest that, at environmental concentrations, ABs do not have an effect on resistance selection. Instead, we emphasize the significance of ARG transport and highlight the relevance of wastewater particles. In 10 L batch reactors, natural river sediments were exposed to a mix of ABs (5 μg/L) or wastewater particles (150 mg/L). Over a period of 2 months, in which ABs were continuously spiked at high concentrations (100 times above environmental concentrations), ARG abundances did not change in sediments. However, after addition of wastewater particles (one single spike), ARGs increased in bulk waters and in sediments by 1 to 5 log units. Interestingly, within 2 months, ARG abundances did not remain elevated but instead returned to their initial concentrations. We observed reduction rates of up to 10^{10} gene copies per litre per day in bulk waters within the first week after addition of particles. These losses can only partly explain the high ARG concentration shifts observed in sediments, hence, strong degradation processes must be involved. ARG concentration shifts in downstream environments are probably caused by a continuous import of ARGs from WWTPs. If this import is removed, concentrations are likely to decrease again.

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A novel method for determining effect concentrations that select for antimicrobial resistance

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Antimicrobial resistance is one of the most significant threats to modern society. Use, misuse and overuse of antibiotics clinically and in the community; in agriculture and in aquaculture results in antibiotics and antibiotic resistant bacteria being released into the natural environment. Environmental concentrations of antibiotics are very low (ng/L range), but recent studies have shown that these concentrations may be sufficient to select for antimicrobial resistance. Currently, environmental risk assessment of it terms of selection for antimicrobial resistance is not performed. This is because there is no ecotoxicological assay with selective endpoints (ie which determine concentrations of antibiotics that select for antimicrobial resistance). This work uses a previously published, qPCR method for determining effect concentrations of antibiotics in complex bacterial communities to validate a novel, rapid growth-based method developed in this study. Spatiotemporal inocula and culturing condition effects were also explored. Results show that standard ecotoxicological assays are not always protective against resistance selection, and selection for antimicrobial resistance could be occurring in particularly impacted environments. Predicted no effect concentrations (PNECs) derived using the qPCR and growth-based methods are highly comparable. Therefore the growth-based method is promising new tool to enable rapid environmental risk assessment to protect against selection for antimicrobial resistance.

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Identifying Hotspots of Resistance Selection from Antibiotic Exposure in Urban Environments around the World

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To understand the impacts of antibiotics on resistance in the environment and the subsequent risks to human and animal populations, it is essential to understand the concentrations of these molecules in the environment. The levels of exposure and the types of antibiotics will vary significantly in different regions of the World due to differences in pharmaceutical availability, wastewater treatment connectivity and infrastructure, regulation and disease pressure. This study was therefore performed to understand the level of exposure for antibiotic compounds in urban aquatic environments around the globe in order to identify regions where environmental exposure to antibiotics could be contributing to the AMR problem. River water samples were obtained from 517 urban locations in 61 countries and then analysed for thirteen antibiotics using LC-MS-MS. Mean concentrations of antibiotics for each river system were compared to predicted no effect concentrations for resistance proposed in the literature to establish the level of risk. Data are now available for 31 of the countries with data for the other countries due by the end of 2018. Highest total antibiotic concentrations (3153 μg/l) were observed in Pakistan with seven of the thirteen compounds being detected. The most frequently detected compound was ciprofloxacin followed by trimethoprim and sulfamethoxazole. Concentrations of antibiotics in 19 of the 31 countries were below $PNEC_{Resistance}$ values indicating that environmental antibiotic exposure from rivers may not be a great contributor to the AMR problem in these countries. Rivers in Austria, Belgium, Cyprus, Iraq, Laos, Malaysia, Nigeria, Pakistan, Portugal, UK, USA had mean concentrations of at least one antibiotic above the $PNEC_{Resistance}$ indicating that resistance selection is possible in these regions. The compound of greatest concern was ciprofloxacin which was found to pose a potential risk in seven of the 31 countries studied so far. Our data analysis is ongoing to extend the range of countries investigated and to identify the environmental, technological and socio-economic drivers of exposure to these molecules as well as areas at risk within each river system.

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The role of wildlife in the environmental dispersal of antimicrobial resistance in agricultural areas.

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Antimicrobial pharmaceuticals are increasingly used in modern society, both for humans and animals. As a consequence, the prevalence of bacteria exhibiting resistance to these pharmaceuticals is increasing concomitantly, potentially having major impacts on the (future) effectiveness of antimicrobial products. A potentially major source of antimicrobial resistance (AMR) and antimicrobial resistance genes (ARGs) is related to agricultural practices. However, little is known about the environmental fate of ARGs, e.g., how they disperse, how long they persist, and what are the environmental reservoirs. In this presentation, we will provide details on the role of small wild mammals in the dispersal of ARGs in The Netherlands. Individuals of five species (white-toothed shrew (*Crocidura russula*), wood mouse (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*), house mouse (*Mus musculus*) and common vole (*Microtus arvalis*)) were trapped at farms that differed in their usage of antimicrobial pharmaceuticals. Faecal samples collected from the trapped individuals were used to quantify the occurrence of ARGs. Different approaches were followed to identify differences in ARG prevalence among species and among farms with different agricultural practices. First, the species-specific microbial composition of faeces was established using Illumina sequencing of barcoded 16S rRNA gene fragments. Compared to the other species, white-toothed shrews had a clearly different microbiota composition. With the shrews excluded, Principal Component Analyses clearly ordinated the other species; bank vole and wood mouse were generally most different. Shrews had the highest diversity in their microbiota profiles; both vole species had the lowest. Differences between farms were less apparent. These results suggest that differences in the microbiota profiles in small mammals are related more to difference among species (e.g., diet preferences) and less to differences between sites. The occurrence of ARGs in the faeces was analysed with detailed shotgun metagenome sequencing of samples pooled per species and collection site. This pooling strategy was used to obtain enough starting material and was feasible because variation in microbiota profiles within species was much lower than between species. We will present details on the species specific diversity of ARGs in combination with gene maps and taxonomy ARG networks, all in relation to agricultural practice.

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Development of a dose-response model for assessing risks from antibiotic resistant E. coli in agricultural water reuse

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Several recent studies have identified antibiotic resistant bacteria (ARB) and residual antibiotics in the effluent of wastewater treatment plants (WWTPs). This is especially true of WWTPs that accept effluent from hospitals or pharmaceutical manufacturers. Moreover, in addition to ARB discharged directly into the environment, the residual antibiotics in the effluent also exert a selection pressure and promote the buildup of additional ARB. This scenario is especially problematic when treated wastewater is used for agricultural irrigation in the effort towards water reuse. There is a potential for the development of infections that do not respond to antibiotic treatments. We here develop a dose-response model taking into account the fraction of antibiotic resistant *E. coli* in the treated wastewater in order to understand the magnitude of the threat. In this model, we assume that in the absence of antibiotics, the ARB does not possess increased virulence. This means that the same model used for the antibiotic sensitive bacteria applies for the resistant strain. However, in the presence of antibiotics, through co-ingestion of residual antibiotics and ARB with water or food, the ARB will survive and out compete the sensitive strains. Under such scenarios, the health outcome depends on three factors: the initial bacterial dose of ingestion, fraction of ARB and the concentration of antibiotic. The key contribution of this study is a dose-response model founded in stochastic growth kinetics that accounts for these three factors in determining the health outcome. We

demonstrate that the new model outperforms the existing dose-response model for *E. coli* infection in human trial studies. Predictions of human health outcomes are also made in the context of residual antibiotic quantities utilizing kinetic data from *in-vitro* experiments of the ARB to inform the dose-response model.

Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring New And Novel Approaches (II)

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EpiSuite prediction models: suitable for natural toxins?

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Screening-level assessment of natural toxins in drinking water in Europe is needed. Many potentially harmful toxins have been identified, but there is a lack of experimental measurements of their environmentally relevant partitioning properties (Bucheli. *Environ. Sci. Technol*., 2014). QSAR models can be used to estimate their partitioning, persistence and mobility in the aquatic environment, but the results should be interpreted carefully. Prediction of physico-chemical properties of interest are available as model outputs from QSAR models in EpiSuite. The training set of the models comprise a database of measured properties of anthropogenic organic pollutants. Natural toxins represent a completely different set of chemicals and hence, the applicability domain of the models is not established (Sahigara et al. *Mol. Basel Switz*., 2012). We extracted the training sets for the EpiSuite QSARs for biotransformation by fish (kM) and octanol/water partition coefficient (Log Kow) and calculated the Euclidean distance (ED) of chemicals from the centroid of the training set chemicals for chemicals in the training set and over 1500 natural toxins found in Europe. Even though the majority of the natural toxins lie within two times the average ED of the training set, comparison with recent experimental values of some of the toxins reveals an overestimation of Kow for polar molecules, among others. The lack of correlation would indicate that ED is rather a qualitative measure and cannot be used to assess the error range. Quantum chemical models, such as COSMO-RS, may be useful to bridge this discrepancy; they do not need a training set database because they are based on first principles (Klamt and Schüürmann. *J. Chem. Soc. Perkin Trans.*, 1993). Comparison with values obtained by other models are showed and discussed critically. Keywords: QSAR, natural toxins, applicability domain, COSMO-RS

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Use of time-weighted average exposure values in the chronic aquatic risk assessment

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In the risk assessment for aquatic organisms, by default the maximum exposure concentration is used. In the chronic aquatic risk assessment for Plant Protection Products (PPPs) in Europe, the time-weighted average (TWA) exposure may be used as refinement, when certain conditions are fullfilled. Indeed in some regions this is the default exposure expression. However, recently the application of TWA-based exposure values has been challenged in the EU, and instead the more conservative maximum exposure estimate is favoured. One concern is that effects may be driven by the magnitude of exposure over the short-term resulting in effects, which would not occur following equivalent TWA exposure at a lower level for a duration. For endpoints related to survival, this line of argumentation perhaps seems intuitively reasonable, that the absolute magnitude of exposure may be important in determining effects. However, the question arises whether or not this might also apply to other endpoints, such as growth and reproduction that typically more relevant to chronic risk assessment. Therefore, we explore the use of the TWA approach as an exposure refinement and whether it retains conservatism afforded at lower tier assesments. It was found that longer exposure at a lower level resulted generally in similar or greater effects than short but higher exposure. This was for endpoints relating to growth (frond number, fish length or weight) and production of offspring (no. of neonates/female *Daphnia*). Since for sub-lethal endpoints similar or higher effects were estimated at longer than at shorter exposure durations, it is expected that the use of TWA exposure estimates will be sufficiently conservative as an exposure measure in the aquatic chronic risk assessment.

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EnviroTox Database: Overview and Applications

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Flexible, rapid, and predictive approaches that do not require the use of large numbers of vertebrate test animals are essential, as the chemical universe remains largely untested. Development of robust new approach methodologies (NAMs) and non-testing approaches requires the use of existing information via curated, integrated datasets. A large, diverse dataset was developed from a range of sources, with harmonization and characterization steps to ensure that the information could be effectively organized and mined. The resulting EnviroTox database (www.envirotoxdatabase.org) contains 91,217 aquatic toxicity records representing 1,563 species, and 4,016 unique chemical CAS#s. Chemical-specific information is linked to each record and includes physical chemical information, chemical descriptors, and mode of action classifications. Toxicity data is associated with the physical chemistry data, mode of action classifications, and curated taxonomic information for the organisms tested. The EnviroTox platform also includes three analysis tools: a predicted no effect concentration (PNEC) calculator, an eco-TTC distribution tool, and a chemical toxicity distribution (CTD) tool. This presentation will provide an overview of the EnviroTox Database and examples of how the database and its associated tools can be used to inform chemical evaluation and alternative method development. The first example demonstrates how the database and tools can be used in a water quality criteria and environmental quality standard application for organochlorine and acetylchlolinesterase pesticides. Records from the EnviroTox database were used to derive threshold values based on PNEC distributions for several pesticide classes (AChE inhibitors and organochlorines) and compared with existing regulatory criteria and discussed. Initial analysis of all records in the database classified as AChE inhibitors by ASTER (10,135 records for 176 chemicals) shows a $PNEC_{0.05}$ that generally agrees with several of the accepted regulatory criteria values. A second example explores how the NAMs can be effectively evaluated against existing acute and chronic data, using compounds classified as neurotoxic. In this case, 14,657 records on 263 substances classified as having neurotoxic modes of action (by ASTER) allows comparison of trophic level-sensitivity. In this case, invertebrates are 2 orders of magnitude more sensitive than fish and 4 orders of magnitude more sensitive than algae.

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Predictive Weight of Evidence (pWoE) for Alternative Testing Systems

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The use of alternative (non-animal) tests as a proxy for the effect of chemicals on laboratory animals requires quantitative understanding of what individual test, or test combination, is sufficiently predictive for the toxicological endpoint at concern. In general, this problem is one of supervised statistical learning, supervised because we have a golden standard to quantify predictive success. Here, we are particularly interested in only a few tests, say 2 or 3, with a binary (two-valued) outcome, in combination with a binary response. The training sets involved are limited as well, in the order of 300 or so, often much less (100+). We have applied logistic regression to model the predictive strength and agreement of the alternative tests. However, instead of assessing overall goodness-of-fit, we have developed a predictive measure of Weight of Evidence (pWoE), which is proportional to the log odds, or log DLR (Diagnostic Likelihood Ratio), of the logistic regression. Here we take common logarithms, multiplied by 10 to obtain convenient and interpretable information units (known as *deciban*). A pWoE between +1 or –1 is considered as insignificant. Mathematically, pWoE equals the linear term in the logistic regression, corrected for the imbalance of positives and negatives in the response. Thus, we can estimate pWoE for each binary test combination, which is much more interesting than overall predictive fit. With the aid of bootstrapping, one estimates the uncertainty of pWoE values. Some test combinations then turn out to be more informative than others, and if test outcomes are in conflict, we can quantify the pWoE and its uncertainty of such combinations. For the case of three common tests for human patch skin sensitization, we show that two, or more, test-minuses always implies a response minus, but a complementary rule for plusses reveals a mixed response.

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Development of a Bayesian network model for predicting fish acute toxicity from FET data: a probabilistic weight-of-evidence approach

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Reduction of animal testing, wherever possible, is required by legislations such as the EU Directive 2010/63/EU. Fish Embryo Toxicity (FET- OECD TG236) testing has been proposed to be an alternative to the juvenile acute fish toxicity test (AFT- OECD 203). However, FET data are not yet accepted as a replacement to the AFT test for certain regulatory purposes such as REACH. The European Chemicals Agency (ECHA) recommended that a weight-of-evidence (WoE) approach was needed for FET data before it could possibly be used in place of AFT data. Therefore, a Bayesian network (BN) model has been developed to incorporate multiple lines of evidence in combination with FET data in order to predict AFT. Bayesian networks are increasingly being used in ecological risk assessment because they can integrate large amounts of data and other information sources to produce discrete probability distributions, and predict the probability of specified states. The objectives of this study were: 1) To develop and evaluate a BN model for predicting toxicity of substances to juvenile fish from embryo toxicity data in combination with other relevant information; 2) To apply the BN model in a WoE approach which can support replacing juvenile fish toxicity testing with embryo toxicity testing. \n

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Panel discussion

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Natural Resources in LCA: Extraction, Processing and Dissipation of Metals, Minerals and Plastics (II)

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Presenting the Plastic Leak Project: A pre-competitive initiative to harmonize plastic metrics
L. Iten, Quantis; A. Ernstoff, Quantis / Quantitative Sustainability Assessment; L. Peano, C. Dubois, M. Zgola, S. Humbert, Quantis; J. Boucher, Shaping Environmental Action & University of Applied Sciences and Arts Western Switzerland
Within the context of circular economy, plastic proves to be a promising material. However, in order for the material to achieve full circularity, leakages of both micro- and macroplastics have to be prevented. As the effects of plastic in the environment on human and environmental health have yet to be examined and for now remain unknown, the pressure to act quickly is high. A careful and robust quantification of the loss streams is needed to identify priority areas for meaningful actions. If this step is ignored, mitigation efforts could be focusing on minor leaks or worse, lead to unintended consequences on the environment. A major research gap has been identified as the projects conducted so far collectively lack of adequate leakage metrics, robust supporting methodologies and lack of a connection to life cycle thinking.\nQuantis, Environmental Action along-side stakeholders from both the public and private sector propose the Plastic Leak Project (PLP). The PLP will define methodological guidelines, deliver sector-specific default data and conduct case studies. The guidelines to quantify plastic leakage are suggested as a global reference on the topic, supported by recognized experts and top international organizations, many of which are already engaged as advisors and stakeholders. The delivered default data will be split in three categories: loss rates (e.g. tire abrasion), release rates (e.g. efficiency of waste water treatment plants) and activity data (plastic waste generation along the supply chain and country-specific end-of-life/recycling scenarios). The case studies will serve to test the guidelines and to leverage the work towards the identification of the hotspots of key area interventions to close the plastic tap. The assessment and prioritization of these actions will also be put in the a life cycle context and carbon footprint will also be assessed.\nThe PLP will add unique value as it connects the marine litter issue with existing environmental assessments. It thus provides a framework that allows for a holistic approach to the plastic leakages that allows to close the tap while avoiding the shift of environmental burdens. The PLP can thus be seen as the compass that leads to a circular plastics economy.

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Exploring the accountability of marine litter generation at the life cycle inventory level
E. Sanye-Mengual, S. Nessi, S. Sala, R. Pant, European Commission - Joint Research Centre / Bioeconomy unit
Marine litter (or debris) is the "waste created by humans that has been discharged into the coastal or marine environment". Among marine litter, plastic marine litter is arising as an important environmental pressure and urgent policy matter due to its long durability and large presence (60-90% of marine litter). Some authors have estimated the global generation of macroplastics and microplastics as well as the potential impacts to the marine environment, biodiversity and human health. Resulting from the "Medellin Declaration on Marine Litter in Life Cycle Assessment and Management", the life cycle assessment (LCA) community is putting an effort on developing methods to address marine litter. Plastic marine litter encompasses macroplastics and microplastics (both primary and secondary), depending on litter size and origin. Macroplastics and microplastics can be emitted to the environment at any stage of the life cycle of a product due to direct or indirect actions, leading to an emission to the marine environment. This contribution aims at defining a framework to quantify the generation of macro- and microplastics at the life cycle inventory (LCI) level to set the basis for an indicator addressing marine litter in LCA. The proposed framework includes two indicators: Cumulative macroplastics generation (CMaG) (kg) and Cumulative microplastics generation (CMiG) (kg). CMaG can be estimated by applying product-specific marine litter rates (MRL) to the mass of each product in the LCI. Estimated MRLs are being calculated by product type and world region as the ratio between the amount of product littered (based on beach and marine counts) and the amount of product consumed. CMiG can be estimated by accounting for the primary and secondary microplastics generation along the life cycle of products. The main sources considered in this framework are plastic pellets (MiG_p), tyres (MiG_t), marine coatings (MiG_{mc}), road markings (MiG_{rm}) and synthetic textiles (MiG_{st}). Bottom-up estimations are based on literature and adapted to the LCI of products in relation to the mass of products and transported distance, mainly. Cosmetics and dust were excluded as primary microplastics sources. This contribution expects to (a) develop a framework to account for marine litter in LCA at the inventory level and (b) apply it to case studies of plastic products for its verification. The developed framework is also expected to be applied at a larger scale, e.g., EU-28 consumption.

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Marine Impacts in LCA (MARILCA): a framework for marine litter life cycle impact assessment
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Environmental impact pathways from marine litter are not yet covered by life cycle impact assessment (LCIA) methods, potentially compromising the robustness of decision-support provided by LCA. For example, replacing all plastic bottles by glass would indeed reduce marine litter but increase other impact categories, such as global warming, and comparing these trade-offs is not possible if those impacts are not included and characterized. Filling this research gap is a high priority for the LCIA research community, as shown by the recent Medellín Declaration, which specifically calls for LCIA model development to account for potential damage caused by marine litter. Here, we present the output from Phase 1 of the MARILCA (Marine Impacts in LCA) working group process; a framework for developing LCIA models for, and illustrating the different impact pathways associated with, marine litter. The MARILCA working group operates under the UN Environment Life Cycle Initiative and with the Forum for Sustainability through Life Cycle Innovation (FSLCI) as a communication partner, the overall objectives of which are presented in a complementary poster presentation. A detailed mapping of possible impact pathways is provided, with guidance on the most important ones from an inventory perspective. Our developed framework links the generation of waste with potential damage to three areas of protection (AoP) namely ecosystem quality, human health and (the proposed AoP) natural heritage by describing mechanistic steps along impact pathways. We recommend development of material-, spatially- and size-differentiated LCIA models including details on fate, exposure and effect processes. Fate processes include those related to waste management (or mismanagement), transport in the environment, and material degradation. Together, the exposure and effect processes describe the potential consequences of marine litter on humans and ecosystems. The framework is centered on plastic litter impact pathways and is expandable to cover other materials constituting litter in the marine environment e.g. metals, glass, organic materials. Overall, we present the basis for harmonised LCIA model development that can first cover the predominant impact pathways and step-by-step generate a complete and coherent damage-level LCIA model for marine litter.

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Development of characterization factors for the assessment of marine litter impacts in LCA
P. Saling, BASF SE / Sustainability Strategy; L. Gyuzeleva, University of Freiburg; K. Wittstock, V. Wessolowski, BASF SE; R. Griesshammer, University of Freiburg
Plastic pollution in our marine environment is taking place on a staggering scale with up to 12 million tonnes of new plastic waste entering the ocean each year. The main impacts of plastic debris on marine life are through entanglement or ingestion often having fatal consequences to individual organisms. Marine litter has been identified as one of the main drivers of biodiversity loss in marine environments, but there is still a high degree of uncertainty related to this issue. There is a growing body of research often focusing on the effects of plastics on individual marine organisms. Now that some of the effects and risks related to

marine debris have been identified, it is important that these impacts on the environment are measured in a systematic and quantitative way. Plastic debris impacts can successfully be implemented in life cycle assessment (LCA) and be measured along with other environmental impacts of products and processes. Today only a limited number of LCA impact categories are related to the marine environment and there is a general lack of focus on marine environments and particularly plastic debris. In the present study we assessed the impacts of certain selected polymers on the marine environment based on a comprehensive literature review and classified substances into different groups and impact categories. Based on these results we developed a characterization factor model for potential embedding into life cycle impact assessment models.

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Plastic waste in water in Ecological scarcity 2103: Development of eco factor and its relevance in packaging LCA
T. Kägi, G. Roberts, F. Dinkel, Carbotech AG
Based on the publicly available data on the amount of plastic entering the water, an ecofactor for plastic emission, in water was determined for the Ecological Scarcity Method 2013 (Frischknecht & Büsser Knöpfel. After establishing the ecofactor, the second step was to determine the average proportion of plastic packaging or other plastic products that end up in the water based on substance flow data. With around 800 eco points (UBP) / g of plastic waste in water, the ecological factor is considerably higher than, for example, the ecological factor for CO2 (0.46 eco points / g CO2). The application to plastic products shows that taking this ecofactor into account leads to a significant increase in the environmental footprint of packaging. This is illustrated on the following example: If a packaging material (e.g. PET bottle) ist completely disposed of in water systems (worst case), the plastic waste in water contributes about a hundred times more to the environmental footprint than all other processes in the life cycle of the packaging material together. In average, we assume, based on Maier-Borst u. a., (2018) and Seyler u. a., (2016), that about 0.3% of a packaging material is disposed of in the environment. Regarding typical packaging materials this contributes about one forth to more than one third of the environmental footprint of a packaging material. (More results will be shown in the presentation). While the current flow and normalization flow is based on relatively reliable data, the critical flow is currently chosen arbitrarily. However, in the current political environment, we consider a 50% reduction to be a plausible target. The treshold value could be even stricter (which means a even higher eco factor). The introduction of the ecofactor for plastics into water leads to a substantial increase in the environmental footprint of plastic-based products, as soon as even a few per mille of them are disposed of into the water. This has a particularly relevant impact on the conclusions of studies comparing plastic products with other products to the detriment of plastic products. Many life cycle assessments about packaging materials are likely to arrive at different conclusions if this factor is taken into account.

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When 'dissipative use' leads to an environmental impact: the case of space missions related impact on the orbital resource
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Adopting a purely anthropocentric view, **the near-Earth orbits** where space activities take place can be encompassed as a part the '**Technosphere**'. In such case, orbits can be considered as an asset of natural resources supporting human activities. **Considering the whole life-cycle of a commercial space mission, the dissipation of the materials occurs in a systematic way after their use phase in orbit**. All the objects launch in space are dissipated in two way: or by staying 'out' of the natural environment once they are non-functional or with direct emissions in the atmosphere caused by the burning during the re-entry stage. In addition, a dissipation of mineral resources could be identifyed dealing with the combustion of the solid propellant during the ascending phase of the rocket. The resource dissipated within the orbital environment leads to a raising sustainability concern: 29,000 human-made objects larger than 10cm are orbiting the Earth but only 6% are operational spacecraft. Space debris is today a significant and constant danger for all space missions. **It is worth noting to note that the current orbital environment is the result of past and present *dissipative use* of the aerospace materials** spread mainly after the completion of the mission or after a collision with a debris. In the same way, the future state of the orbital environment will be directly linked to this dissipation phenomena regarding the contribution of each new mission scheduled. However, the impact linked to the *resource dissipation* is never taken into account in the current environmental LCA studies which adopt a *cradle-to-launch pad* approach. Therefore, a focus on the dissipated resource within the orbital environment seems today particularly relevant to ensure the sustainability of the outer space activities. Hence, our challenge is to integrate debris related impacts within the LCIA step to broaden the scope of LCA for space systems. First, we present a general framework to integrate the impact of the 'dissipative use' on the orbital resource. Then, we propose a set of Characterisation Factors (CFs) as a way of measuring the impact of the previous dissipated resource on the system under study. A case study will be presented to demonstrate the applicability of the methodology. The assessment of the potential trade-off occurring in term of environmental impacts on both the Earth and orbital environment will be discussed, comparing several Post-Mission Disposal (PMD) scenarios.

Difficult to Test Substances and Wastes: Regulatory Status, Testing Challenges, Interpretation of Data and Fulfilling Information Requirements

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Environmental effects of data-poor metals: the example of palladium
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The Platinum Group Metals ('PGMs') are poor in ecotoxicological data, inhibiting a proper assessment of the ecotoxic potential and risks. Driven by EU REACH, industry has been generating a basic dataset of acute aquatic toxicity data. For palladium, effects are observed at low μg Pd/L range. The test data also suggest varying toxic thresholds depending on the molecular structure of the Pd test compound. An extensive literature search was performed and all reliable data were combined in a datamatrix. The published literature only investigated the ecotoxic response of palladium chloride A testing program was established to generate ecotoxicity data for Pd compounds with variable coordination structure and for most critical test species, in order to compare test data for these Pd compounds and derive a robust set of ecotoxicity threshold values. Algae are identified as the most sensitive test species to Pd, with EC50 values as low as 3 μg Pd/L. However, algal EC50 values are up to 10+ times higher for different Pd compounds, and 100+ fold for fish. PGMs are known to have a strong coordination behaviour to (in)organic ligands in aquatic media. As such, and in contradiction to most data-rich metals, the presence of Pd as free Pd^{2+}-ion in solution is very low. By comparing the responses observed for various Pd compounds, it will be checked if toxicity differs between coordination complexes, and if there is a general predictor of toxicity. One of the tools that will be used is the QICAR (Quantitative Ion Character-Activity Relationships) model that is under development and will be available at the end of 2018. Taking into account all the above, an appropriate approach will be defined to derive environmental toxicity threshold values. The learnings from this work will be applied in a next step to other PGMs like Platinum, Rhodium and Ruthenium.

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Interpretation of algal growth rate data for aquatic classification of rapidly degrading substances; Zinc pyrithione as a case study.
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Algal growth rate inhibition at 72 hours, expressed as 72h ErC50s and ErC10s, is the preferred algal endpoint for aquatic classification according to the CLP regulation (1272/2008) and OECD 201 is the preferred test guideline. However, the CLP regulation and OECD 201 are neither well adapted to rapidly degrading substances nor address the diversity of taxa useful in assessments. The use of 72h ErCx values based on mean measured concentrations of rapidly degrading substances can significantly overestimate toxicity. Characterizing acute toxicity of rapidly degrading substances with a 72h ErC50 can also be inconsistent with the definition and concept of acute toxicity in the CLP regulation, which is an effect during a short term exposure relative to lifecycle. The use of shorter study durations could be a solution for rapidly degrading substances, but the first validity criterion in OECD 201 relates to biomass increase and may not be fulfilled in less than 72h, particularly for slower growing taxa than those described in OECD 201. These points are illustrated with data from a recent study conducted with zinc pyrithione (ZPT) in the marine diatom, *Skeletonema costatum*. ZPT is photolytically unstable and rapidly degraded in algal toxicity studies due to required lighting conditions. Concentrations dropped rapidly to less than 50% of initial concentrations by 24h, then continued to decline to about 10% of initial by 72h. The 72h ErC50 based on mean measured concentrations from this study is less than an initial concentration that caused no discernible growth rate inhibition at any time point. *S. costatum* growth in the controls was constant, consistent and exponential but not reaching 16x increase before 72h. Therefore, according to the guideline, shorter study durations were not considered valid. Similarly, OECD 201 does not allow for use of nominal concentrations in this case. The conclusion that the 72h ErC50 is the most relevant for classification in this situation, despite being lower than an initial concentration showing no effects, is neither reasonable nor consistent with the principle of acute aquatic toxicity. For rapidly degrading substances, a better approach to assessing acute and chronic algal inhibition (toxicity) is needed. Options could include use of nominal concentrations for toxicity estimates, use of recovery tests to assess algicidal concentrations, shorter test durations and specific considerations of the taxa under evaluation.

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Determining bioconcentration factors of constituents of essential oils using in-vivo benchmarked dietary exposure studies
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University / Environmental Research Institute; M. McLachlan, Stockholm University / Environmental Science and Analytical Chemistry (ACES); G. Horlitz, Stockholm University, ACES / ACES; M. MacLeod, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES

Persistence (P), potential for bioaccumulation (B) and toxicity (T) are established criteria for the (regulatory) assessment of chemicals. While PBT testing methods are standardised for single component chemicals, there is a lack of standard methods for the assessment of complex mixtures. An example are essential oils - fragrance materials that are registered as natural complex substances (NCS) under the European REACH legislation. NCS are widely used, but their potential environmental hazard is not fully understood. Models predict that many essential oils are likely toxic for aquatic wildlife and persistent. They are also suspected to be bioaccumulative, but the available predictions for "B" are scarce and only "moderately reliable" (ECHA website). A challenge for determining the bioconcentration factor (BCF) of essential oils is that it cannot be readily measured using a standard flow-through experiment. The OECD 305 guideline provides the option to determine the BCF using a measured depuration constant (kT) from dietary exposure and an estimated uptake rate (k1). However, this approach has been criticised for the variable quality of k1 estimates based on the used models - and hence potential limited reliability of the resulting BCFs. To assure the quality of BCFs from dietary exposure, we used well-characterised reference chemicals (chlorobenzenes (DiCB, TrCB, PeCB), PCB3, PCB52 and musk xylene) for which the determined BCF were compared with literature data. Using our previously reported benchmarking methodology, we determined the BCF of key components in cedarwood and pine oil as well as the reference chemicals in a single dietary exposure experiment on rainbow trout. Using hexachlorobenzene (HCB) as benchmark, the BCFs for the main components of pine oil, cedarwood oil, and the reference chemicals were between 500 L kg^{-1} (Bornyl Acetate) - 55200 L kg^{-1} (PCB52). The apparent BCF value for HCB was 2244 L kg^{-1}. The determined BCF of the reference chemicals were within the same order of magnitude of reported literature values and resulted in the same assessments regarding "B". Using benchmarking, we established a robust method for the measurement of kTs of NCS in fish with a single dietary exposure, as well as establish a quality control for the resulting BCFs.

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Biochemical biomarkers opposite effects - characteristics, meaning and methods for proofing

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Biochemical biomarkers are widely used as sensitive and early warning signals of exposure to different kind of pollutants. Some biomarkers or their combinations are an indicator for the presence of a specific pollutant or group of contaminants in the environment. Biomonitoring procedures are usually based on the comparison of measured values of biomarkers in field or exposed organisms with control/intact organisms or benchmarks. The most common interpretation of biomonitoring using biochemical biomarkers consists of simple rules based on the decrease/increase of some enzyme activity or presence/absence of some chemical compound in test organism. Due to such expectations, the events of the opposite effects are largely neglected. Within this report, the data contained in recent literature, as well as the results of our experiments, are compared and presented. In addition to the usual type of opposite effect (hormetic like effect), there are some evidence of the existence of so-called double or two-way hormetic like effect. A significant number of scientific papers with opposite biochemical biomarker effect measured was found. However, only in a few papers (less than 1%) such results were also described in the text. The results of our experiments showed that the occurrence of opposite effect is not rare, only in some cases difficult to prove, mainly as a consequence of an insufficient sample size that would allow detection of subtle changes in biomarkers. If, at the highest dose of effectors (that inhibit some system element), organisms are still vital, it is possible to expect an onset of additional mechanisms able to compensate the inhibited system element resulting with a specific response curves. On this curve it is possible to recognize four asymptotes or levels: normal, hormetic, minimal and compensatory level. The most common opposite effects are hormetic like effects, while recently double or both-sides hormetic effects are described and proven. The better knowledge of low-dose toxicology, as well as a knowledge of phenomena that differ from the generally accepted dose-response models are very important because opposite effects can also be harmful to organisms. Moreover, the techniques and methods that could detect and prove the effect in low-dose range could be used as tools for the detection of early toxic effects. This is particularly important for the biomonitoring of environmental pollution and for an environmental risk assessment.

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Evaluation challenge of waste toxicity at oil extraction

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Increasing oil extraction activity all over the world, imperfect technologies of oil production leads to formation of different wastes, containing petroleum hydrocarbons. Drilling cuttings are one of the oil extraction wastes, consisting of solid rock material and residuals of drilling fluid. Drilling cuttings could be landfill or utilize to recultivants. Being contact with environmental components, drilling cuttings or their composites, could impact the environment and remain toxic effect for many years. Ecotoxicological tests in combination with chemical studies could be the most suitable approach to assess the ecotoxicity of oily wastes and prevent contamination risk. We studied the ecotoxicity of drilling cuttings and the composites, formed on the base of drilling cuttings, in order to find the safety admixture and their ratio to return a waste in transformed state into environment. Five different admixtures and the wastes were tested. Drilling cuttings were mixed with sand to improve physical characteristics, peat and kieselguhr (diatomaceous earth) to increase the sorption capacity, phosphogypsum to regulate the acid medium of the mixture and cement to give mechanical robustness. We applied the bioassay to assess the toxicity of drilling cuttings and the formed composites. We evaluated the initial concentration of contaminants in aqueous extracts from wastes and compositions that may immobilize 50% of crustaceans and infusorians, but available approaches of bioassay have significant restrictions for implementation. The tested samples contained high level of water soluble salts (up to 50 g kg^{-1}).The eluate and direct phytotoxicity tests were run, using seeds of mustard (*Sinapis alba* L.) and oat (*Avena sativa* L.). The drilling cutting samples with TPH content higher than 3.0 g kg^{-1} were toxic, then how the composite variants with TPH content less than 35 g kg^{-1} stimulated the plant growth compared with the control. Treatment with kieselguhr and peat allowed sorbing TPH and tracing metals, that approved a positive effect of chosen ingredients and their ratio. The water filtrates characterized by neutral pH (7.4), TPH content between 1.5-3.1 mg L^{-1}. We conclude it is the shortage of appropriate approaches for ecotoxicity assessment of wastes and obtain the solid results. It is highly demanded to work out new methods to evaluate the toxicity of specific waste based on their composition, properties, aggregation and natural conditions of zone they formed.

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UVCB substances: Methodology for substance identification and an endpoint non-specific selection of computationally generated representative constituents

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Keywords: UVCBs, representative sample, hazard assessment, *in silico*

Substances of unknown or variable composition, complex reaction products and biological materials (UVCBs) comprise about 40 % of all registered substances submitted to the European Chemicals Agency. One of the main characteristics of UVCBs is that they have no unique representation. They are usually described by generic substance identifiers such as names of chemical classes, structural formulas, reaction steps, physicochemical properties, or spectral data. Defining an inambigious structural description aims to complete the following two tasks: to allow performing of *in silico* hazard assesment, where structutal information is essential, and to contribute towards fulfilling the requirements of implementaion of any chemical related legislation. It has been acknowledged that one of the obstacles for the explicit structural description of the UVCBs is the large number of possible structural isomers. A methodology, based on the generic substance identifiers, is developed to address this issue. The methodology allows for the coding of constituents, their generation, calculation of important characteristics of UVCB constituents, and selection of representative constituents. Two main formats are developed: Generic Simplified Molecular-Input Line-Entry System (G SMILES) and Generic Graph (G GRAPH). G SMILES is a SMILES-based single line notation coding fixed and variable structural features of UVCBs. G GRAPH allows generation of constituents coded in G SMILES, calculation of physicochemical properties, simulation of reactions, selection of constituents and their storage in a text file. There are two main types of selection of constituents. The first one is an end-point driven selection based on ranges of a characteristic physicochemical property, e.g. log*Kow* values are critical when assessing bioaccumulation potential. The second approach is a statistical selection of the minimum number of generated constituents representing a UVCB. This representative sample is selected in such a way that the structural variability and the properties of concern of the UVCB are approximated within a predifined tolerable error. The aim of the statistical selection is to enable the assessment of UVCB substances by decreasing the number of constituents that need to be evaluated. The procedure, which is shown to be endpoint independent, was validated theoretically and on real case studies.

Your Science or my Science? When the Sides taken on a Research Topic are Impacted by Different Philosophical Positions and Values (II)

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Food contact chemicals and human health: facts and fiction
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Chemicals migrate from all types of food contact articles (FCAs). Which chemicals migrate and at what levels depends on physico-chemical parameters of both the FCA and the foodstuff. Over ten thousand chemicals are intentionally used in the manufacture of FCAs, and many more are present in finished FCAs as non-intentionally added substances (NIAS). EU regulations require a risk assessment for each migrating chemical. But for many of the intentionally added substances (IAS) toxicological data are insufficient, lacking or dated. Many of the NIAS are unknown which means it is not possible to assess their hazard, exposure and risk using conventional risk assessment approaches. Indeed, several food contact chemicals are endocrine disrupters but their hazard characterization is not routinely performed. And many chemicals migrate simultaneously from a finished FCA, indicating the need for mixtures risk assessment which is currently not required. Therefore, novel methods are being used, including computational tools, in vitro bioassays and in vivo approaches. And an enhanced hazard characterization would address today's most prevalent chronic diseases in the human population, by targeting human health outcomes of concern.

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Food Contact Materials – wishes and reality
T. Gude, Swiss Quality Testing Services
Food contact materials and especially their safety assessment are more and more of higher interest. This depends on one hand on the changing views to chemicals in light of the REACH registration and the hereby-linked re-assessment of safety aspects. On the other hands, it depends on the usage of such materials like in food contact materials in terms of environmental aspects combined with a very restrictive view on toxicology and its real impact. Currently every month a new substance – so far widely used in food contact applications – is in the focus and linked to potential toxic effects. This creates a lot of rumour in the value chain, especially in the retailer channel and finally for us, the consumer. Within this, new publications related to toxic effects, it always is forgotten, that materials like food contact materials do have a positive impact on food safety and food waste management. A change of a very effective system cannot be done very shortly, as a longer development phase is needed. Nevertheless, if compounds with proven toxic profile are used they need to be replaced. However this must be based not only on potential toxic effects mainly shown in higher concentrations, it is mandatory that concentrations migrating from materials in light of a relevant exposure showing such toxic effects. Currently it can be observed that the classification of chemicals is mainly based on effects, not on concentration. This procedure leads to a high pressure on companies to replace proven effective food contact material systems by unproven new systems – possibly associated with new problems. A little more patience and a more pragmatic approach might be helpful to deal with food contact materials. One aspect of a pragmatic approach is not only focussing on individual compounds, but also considering full food contact material solutions.

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Risk assessment of food packaging materials: use of data and methodologies in the scientific assessment
C. Roncancio Pena, EFSA / Food Ingredients & Packaging
In accordance with Regulation (EC) No. 1935/2004 on materials and articles intended to come into food, the EFSA's Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) evaluates the safety of certain substances prior to their authorisation for use in Food Contact Materials (FCM) plastics. EFSA provides scientific advice to risk managers, by communicating on risks to the public and by cooperating with Member States and other parties to deliver a coherent, trusted food safety system in the EU. EFSA should remain a forward-looking organisation that possesses the tools to address new and complex risks. Within this perspective and in order to establish the safety from substances migrating from FCM, both toxicological data indicating the potential hazard and the likely human exposure data need to be combined. In case of testing from migration using food stimulants, the use of mathematical migration models has developed significantly in recent years. EFSA is also actively identifying and collecting information regarding new testing strategies that could be applied to the different sectors of EFSA's work, including the use of the weight of evidence approach, evaluation of mixtures and its genotoxicity, publishing guidance documents, to transparently communicate the principles guiding the risk assessment and at the same time supporting the applicants/interested parties when preparing a dossier to be submitted for evaluation.

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Discussion with presenters panel and audience

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Developing Trophic Ecotoxicology to Support Impact Assessment across Ecosystem Boundaries

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Pathways of pollutant transport across the aquatic terrestrial interface (I)
R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment; M.H. Entling, H. Jungkunst, E. Kroener, A. Lorke, University of Koblenz-Landau / Institute for Environmental Sciences; R. Schaefer, G.E. Schaumann, University Koblenz-Landau / Institute for Environmental Sciences; K. Schwenk, University of Koblenz-Landau / Institute for Environmental Sciences
The role of freshwater ecosystems as a source of nutrients, energy and pollutants is a relatively underappreciated research field, compared with the impact of catchment properties on receiving surface waters. Pollutants may reach the terrestrial ecosystems, and thus lead there to an exposure, through a biological pathway, i.e. via emerging insects or aquatic prey consumed by terrestrial predators. Alternatively, an abiotic pathway exists, and aqueous-phase or particle-bound pollutants are transported via flood events to the surrounding terrestrial habitats. Once pollutants reach the terrestrial ecosystem, they may lead to top-down or bottom-up directed effect cascades in the terrestrial ecosystem/food web, yet our knowledge on the effects in the terrestrial recipient system is extremely restricted. In this context, stable isotope analysis is a valuable tool to untangle the complexity of interactions in the receiving ecosystem supporting an informed decision making as part of chemical risk assessment or ecosystem management. This presentation will summarize the state of the art in the field of cross-ecosystems effects of chemical pollution at the aquatic-terrestrial interface.

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Pathways of pollutant transport across the aquatic terrestrial interface (II)
R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschuh, Institute for Environmental Sciences University of Koblenz-Landau / Department of Aquatic Sciences and Assessment; M.H. Entling, H. Jungkunst, E. Kroener, A. Lorke, University of Koblenz-Landau / Institute for Environmental Sciences; R. Schaefer, G.E. Schaumann, University Koblenz-Landau / Institute for Environmental Sciences; K. Schwenk, University of Koblenz-Landau / Institute for Environmental Sciences
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Subcellular metal partitioning in benthic organisms to estimate the proportion of metal trophically available to wild fish
N. Urien, Institut National de la Recherche Scientifique / Centre Eau Terre Environnement; H. Sonnenberg, EcoReg Solutions Inc; L.D. Ramilo, Ecological and Regulatory Solutions Inc. / Environmental Services; P. Campbell, Université du Québec / INRS Eau Terre Environnement; P. Couture, Institut National de la Recherche Scientifique / Centre Eau Terre Environnement
In aquatic environments, metals may pose a risk to benthos and to the fish feeding on them. Previously, it has been shown that subcellular metal partitioning in prey can influence metal availability for trophic transfer. It is generally understood that metals found in organelles and cytosol of prey represent trophically available metals (TAM) to predators, whereas metals associated with the NaOH-resistant granules and cellular debris fractions pass through the gut for direct excretion. The aim of this study was to assess trophically available metals (TAM) in white sucker (*Castotomus commersonii*) prey for As, Cd, Cu, Se and Zn, using a subcellular partitioning approach. For that, invertebrates from different functional feeding groups (*Hyalella azteca*(HA): shredder; *Hexagenia*(HE): gatherer; and *Enallagma*(EN): predator) were collected in a lake located downstream from a metal mining discharge (~40% effluent) and in a reference lake. Subcellular partitioning among "organelles and cytosol" (TAM fraction), "granules" and "debris" was determined after differential centrifugation, NaOH digestion and heat-treatment steps. The amount of metal associated with each fraction was

determined by inductively coupled plasma mass spectrometry. For all metal(loid)s studied, total concentrations were significantly higher in exposed compared to reference invertebrates, with the exception of As, Cd, Cu and Zn in EN, for which no statistical differences were found. Selenium accumulated the most in all organisms (from 15 to 40-fold), followed by Cd (from 5 to 8-fold). The [Cd] in the detoxified granule fraction was higher in exposed HA and HE compared to those observed in reference organisms, whereas [Cd] in the other fractions remained low and constistent between areas. Contribution from the granules in exposed HA and HE was dominant (> 80%) and contribution from the TAM was very low (< 14%).Hence, trophic transfer of Cd via consumption of those invertebrates is notlikely to occur. In contrast, [Se] were systematically higher in the "debris" and "organelles and cytosol". Proportions of Se were similar in reference and exposed organisms for the three subcellular fractions. Contribution from the TAM fraction was about 30% for HA, 40% for EN, and 55% for HE. Given that [Se] were markedly higher in the exposed compared to reference invertebrates, trophic transfer of Se via consumption of these invertebrates is likely to occur, and represents a risk of toxicity for fish.

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Implications of trophic variability on modelling bioaccumulation of persistent organic pollutants in marine Arctic ecosystems

R. Hoondert, Radboud University Nijmegen / Department of Environmental Science; N. van den Brink, Wageningen University / Dept of Toxicology; M. Van den Heuvel-Greve, Wageningen IMARES / Marine Coastal Systems; A.M. Ragas, Radboud University / Department of Environmental Science; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Science

The occurrence of persistent organic pollutants (POPs) in the Arctic has been of constant concern, as they are known to cause adverse effects in organisms at higher trophic levels, including mortality. Despite the banning of many of these compounds, high levels of POPs have been reported in Arctic biota, likely due to a combination of unfavorable chemical properties and northward marine currents. As the Arctic acts as a chemical sink, this system makes an interesting case for bioaccumulation studies, hence the growing interest in this topic. Typically, bioaccumulation of chemicals is evaluated by calculating trophic magnification factors, based on sampled chemical concentrations, requiring an extensive amount of empirical research. Consequently, multiple *in silico* mechanistic methods have been developed to predict bioaccumulation of hazardous compounds in food chains, which have been predominately validated for temperate food chains, due to lack of sampling of biota in Arctic areas and the strong seasonality of trophodynamics in Arctic systems, mainly driven by fluctuations in light and temperature, affecting the community composition and therefore prey availability. The aim of the present study was to obtain a better understanding of how bioaccumulation models perform when subjected to high trophic variability in Arctic ecosystems. Data were collected on trophic positions (TPs) of Arctic species, based on stable nitrogen isotopic values, by an extensive literature search, together with data on POP concentrations and species- and chemical-specific parameters. Bioaccumulation of POPs in the present study was calculated using the OMEGA bioaccumulation model. In each Monte Carlo iteration, trophic levels were re-binned based on the earlier derived TP probability distributions. Bioaccumulation could be modelled for three areas (Canadian Beaufort Sea, Canadian Archipelago and the Svalbard Archipelago), encompassing a total of 60 compounds. Highest efficiencies/lowest RMSEs were calculated for PBDEs, PFASs and PCBs, while the model performed less well for PAHs. Although in the majority of cases, bioaccumulation was slightly underestimated, variability in trophic position of a species showed to have only minor implications for bioaccumulation modelling.

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Contaminated sediments effect on aquatic insects - cross boundary transport of nutrient and contamination

J. Lidman, Department of biology and environmental science, Umeå university / Department of Ecology and Environmental Science; . Berglund, Umea University / Ecology and Environmental Science; M. Jonsson, Umea University / Department of Ecology and Environmental Science

Anthropogenic contamination of aquatic environment can affect insectivorous birds both directly through exposure to contaminants transported by aquatic insects and indirectly by changing the food availability. In this study we used nine lakes with different concentrations of lead (Pb) to assess the effect of Pb on aquatic insect community composition and production and analyzed metal concentration within emerging aquatic insects to estimate the metal flux from the aquatic environment. A comparison between metal concentration in insect larvae and adults was also done to study the effects of metamorphosis on metal exposure risks. Community composition changed with increased Pb exposure but was only observed in the benthic community with proportionally more Trichopterans and less Ephemeroptera when Pb increases. A surprising positive correlation between Pb concentration and Ephemeroptera and Trichoptera abundance was observed which indicates that other factors are influencing the insect community. Metal flux from the aquatic to the terrestrial system by aquatic insects ranged between 65.9â_110.8 μg/m2 during the study period. Metal concentration differed both between insect orders and between adults and larvae within the same insect order, with higher Pb concentrations in larvae and predatory insects. A higher concentration in larvae shows that metamorphosis influence the exposure risk for terrestrial insectivores feeding on aquatic insects. The results clearly show that contaminants in aquatic systems can affect both food availability and exposure risk for terrestrial organisms.

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Agriculture and mining contamination contribute to a productivity gradient driving cross-ecosystem associations between stream insects and riparian arachnids

F.J. Burdon, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment

Stream and riparian ecosystems can be strongly connected via the emergence of aquatic insects, which form an important prey subsidy for a wide range of terrestrial consumers. However, human perturbations that impact coupled ecosystems may propagate across habitat boundaries. Here, I explicitly investigated how stream pollution states influence communities of stream and riparian invertebrates in 37 forested stream types. Previous research has suggested that donor-habitat productivity gradients influence resource flows, and I hypothesised that stream pollution states would strongly contribute to a resource continuum driving cross-habitat connectivity and community composition. Streams receiving acid mine drainage (AMD) supported low levels of benthic insect and riparian arachnid biomass, whereas streams in forest fragments surrounded by agricultural land-uses seemingly were more productive, with greater standing crops of stream and riparian invertebrates than AMD or reference sites. Structural equation modelling confirmed the positive influence of standing benthic insect biomass on riparian arachnids. Human impacts seemingly also increased the concordance between invertebrate assemblages, suggesting that resource flows may affect community structures across habitats. Overall, these findings highlight how human activities (e.g., mining, agriculture) that influence stream pollution states may weaken or strengthen aquatic-terrestrial linkages.

Socio-Economic Analysis in Chemicals Regulation and Policy Making: Current Challenges and the Way Forward

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SEA under REACH: State-of-play and areas for improvement

C. Rheinberger, ECHA / Risk Management Implementation Unit

Socio-economic analysis (SEA) under REACH has matured over the last 5 years or so and several practices have been improved and aligned with economic theory. In this presentation, we will present an overview of the current state-of-play of SEA conducted to support applications for authorisation and restriction proposals. We will draw on experiences from more than 200 uses applied for authorisation and about 30 restriction cases to highlight problems, discuss proposed solutions, and identify areas for further improvements.

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Incentives for truthful reporting in seeking authorisation for using substances of very high concern

D. Slunge, gothenburg university

In order to attain an authorisation for the use of a substance of very high concern (SVHC) within REACH, companies need to prove that the benefits of use are larger than the costs. Due to asymmetric information, it is difficult for the regulator to know if the information about costs and benefits for continued use of SVHCs provided by the companies is correct. Based on a review of the estimated costs and benefits in 114 socio-economic analyses submitted to ECHA we examine if there is evidence of untruthful reporting by companies seeking authorisation. Furthermore, we discuss design options for an authorisation system providing effective incentives for truthful reporting of costs and benefits. One option is an 'SVHC use fee', being a share of the SVHC substitution costs reported by companies seeking authorisation.

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Political Economy of Inclusion on the REACH Candidate List of Substances of Very High Concern

J. Coria, University of Gothenburg / School of Business Economics and Law

The European Union regulation on the registration, evaluation, authorization, and restriction of chemicals - known as REACH- is intended to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of the thousands of chemicals commonly used in the EU. Both the European Chemical Agency and member states can prepare dossiers proposing substances to be considered for inclusion on the Candidate List of Substances of Very High Concern. The registry of Intention (ROI) is a public registry intended to make all interested parties aware of the substances for which a dossier is intended to be submitted. The Registry of Intentions is divided into three separate sections including current intentions, submitted dossiers and withdrawn intentions and submissions. In this study we investigate what types of SVHC are suggested by different countries, and in particular, how the national production of the substances and their toxicological properties can affect the countries' decision

to submit a dossier. Moreover, we investigate what substances for which a dossier has been submitted are finally put forward for inclusion on the Candidate List

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Can SEA be a reliable decision-making tool for proper risk management on chemicals in a safe and circular economy? The metals industry perspective
H. Waeterschoot, Eurometaux
SEA considerations may help in both ex-ante as ex-post evaluations of risk management measures (RMM) and in assessing the optimality of such measures under REACH or CLP. On REACH, SEAs build on risk-based information and the suitability (or not) of alternatives to define the cost effectiveness of different RMMs. SEAs often need to overcome the lack of comprehensive data by using defaults and sensitivity assessments to cover for uncertainties. When communicating the outcome of SEAs for decision-making on the further use of or constrains on chemicals, the challenge is the often-different perspective of stakeholders on effectiveness assessments of RMMs. Examples from the metals sector will show how SEAs on chemicals management can combine different perspectives and help in effective decision-making to reduce risks during the substance's life-stages. Closing the materials loop by recycling and reuse is for metals and inorganics a critical economic and environmental step toward sustainability but can be a challenge too. SEA and Analysis of Alternatives therefore require a wider scope than the manufacturing and use stages. Examples including case studies will show how such broader frame could be established and help scientists and regulators identify need, scope and format of an RMM.

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Impact assessment in SEA: A structured review of 24 REACH registration dossiers
I. Hilber, Agroscope ART / Environmental Analytics
A core component of SEA used in REACH authorisation and restriction processes is impact assessment. In our study we evaluate twenty-four REACH restriction dossiers regarding the methodological setup of the impact assessment. Furthermore, we examine how the outcomes of impact assessment influence the evaluation of one or several risk management options (RMOs) for restricting the substance, and the selection of the most preferred option. In addition, we investigate whether or not the selection of the preferred RMO is objective, transparent, and comprehensible. Our results show that a comparison of impacts focuses on selected impacts only (in particular human health and economic impacts). While this can sometimes be explained by the very specific scope of a restriction dossier and by lacking data, we also observe a lack of approaches for assessing environmental, health, social, and distributional impacts from chemicals use, and we formulate suggestions how this can be overcome.

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SEA experiences from RIVM (The Netherlands)
A. de Blaeij, J.K. Verhoeven, National Institute for Public Health and the Environment RIVM
The presentation elaborates on our experiences and lessons learned with Socio Economic Analyses (SEA) for hazardous substances under REACH and in other regulatory contexts. During the previous years, the RIVM, in its role as a competent authority under REACH and as research institute, has worked extensively in the field of regulatory toxicology and risk management of hazardous substances. SEA can be useful for a variety of risk management issues around substances of concern, both linked to ecotoxicity as human health, and in various stages in the risk management process. Various research projects have been conducted within the framework of strengthening the SEA methodology, such as guidance for the performance of an Environmental Impact Assessment (EIA), Health Impact Assessment (HIA) and risk governance in SEA. In this presentation, we will e.g. discuss the importance of problem definition, scenario definition and scoping in the SEA process and investigate what socio-economic elements can be considered within SEA in the context of chemical legislation.

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Panel discussion with questions from audience

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Criteria and Methods for Identifying Chemicals of Greatest Concern

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The Origin and Evolution of Assessment Criteria for PBT Chemicals and POPs
M. Matthies, University of Osnabrueck / Institute of Environmental Research; K.R. Solomon, University of Guelph / School of Environmental Sciences; M. Vighi, IMDEA Water Institute / Earth and Environmental Sciences; A. Gilman, Sustainable Solutions International; J. Tarazona, European Food Safety Authority / Pesticides Unit
Public concern over the effects of persistent chemicals began in the early 1960s. Since then, significant scientific advances have increased our understanding of persistent, bioacumulative, and toxic (PBT) chemicals and the properties and processes that influence their fates in, and adverse effects on, humans and the environment. In addition to the scientific advances, a number of legal instruments and agreements for global, international, and national identification and control of persistent organic pollutants (POP) and PBT chemicals have been adopted. However, some of the rationales and thoughts that were relied upon when the first criteria were developed to identify and categorize PBT chemicals and POPs have not been carried forward. Criteria for identification of PBT chemicals and POPs have been based upon available data for neutral hydrophobic "reference chemicals", derived under laboratory conditions, and consensus-based policy decisions. Criteria have evolved over the last decades due to the diversification of the protection aims under various national regulatory frameworks and international agreements, advances in methods for estimation of physical/chemical properties, and the identification of chemicals which are non-traditional POPs. Criteria are not defined purely by science; they also are subject to the aims of policy. This paper offers a historical perspective on the development of criteria for PBT chemicals and POPs. It also offers suggestions for rationalization of protection goals, describes some emerging procedures for identification of compounds of concern, and proposes information that needs to be considered when applying criteria to screening and/or evaluation of new chemicals. The genesis of this presentation stemmed from a perceived lack of a full understanding in the scientific and regulatory community of the original intentions and scientific methods available to identify PBT chemicals and POPs. The understanding of the historical background of the definition of PBT and POP criteria and its modification due to the aims of policy is of increasing interest for the chemical risk assessment community. Matthies M, Solomon K, Vighi M, Gilman A, Tarazona J. The Origin and Evolution of Assessment Criteria for Persistent, Bioaccumulative and Toxic (PBT) Chemicals and Persistent Organic Pollutants. Environ Sci:: Processes Impacts 2016,18, 1114-1128.

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The use of PBT assessment criteria in regulatory environmental risk evaluations: Challenges and Opportunities using cyclic volatile methylsiloxanes (cVMS) as a case study
K. Thomas, Global Silicone Council
There is a broad recognition that a risk-based weight-of-evidence (WoE) approach should be used in the context of a PBT assessment to evaluate the risk of chemicals, and that single criterion should not be used to make definitive PBT determinations. Regulatory evaluations in Europe, Canada, and Australia have assessed the environmental risk associated with cyclic volatile methylsiloxane (cVMS) compounds. These regulatory risk evaluations provide an opportunity to highlight key differences in the approaches used to assess the compounds, and to understand the implications of those differences for the outcomes of the evaluations.

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Identification of chemicals of potential concern based on structural similarity
P. Wassenaar, National Institute for Public Health and the Environment (RIVM); E. Rorije, N. Janssen, National Institute for Public Health and the Environment RIVM; W. Peijnenburg, RIVM / Center for Safety of Substances and Products; M.G. Vijver, CML Leiden University / Environmental Biology
There is a strong demand for more attention and improved grip on chemicals of potential concern to address societal discussion on unwanted emissions of potentially hazardous chemicals. This is acknowledged by the European Commission (setting out a long-term vision of a non-toxic environment) as well as by the Dutch Ministry of Infrastructure and Water Management (setting out the ambition to identify so-called "potential-Substances of Very High Concern"). To this end, we developed a methodology that allows identification of chemicals of potential concern based on structural similarity with substances of known concern. The approach uses the similar property principle, which states that structurally similar chemicals are likely to have similar properties. The methodology focusses on substances with Carcinogenic, Mutagenic or Reprotoxic properties (CMR), Persistent, Bioaccumulative and Toxic or very Persistent and very Bioaccumulative properties (PBT/vPvB), or Endocrine Disrupting properties (ED). We analysed the predictive performance of 112 different similarity measures, consisting of a binary fingerprint and a similarity coefficient, for identification of chemicals of potential CMR, PBT/vPvB or ED concern. A dataset of 554 substances with known concerns for CMR, PBT/vPvB or ED properties was used. In addition, 412 control substances that were known to lack CMR, PBT/vPvB and ED properties, were selected. The best combination of fingerprint and similarity coefficient showed a high predictive performance with a balanced accuracy of 0.82 for CMR, 0.95 for PBT/vPvB and 0.99 for ED. This methodology will be implemented in a software tool that can be used to assess whether a new or existing substance, for which no toxicity data is available, is of potential concern based on structural similarity with chemicals of known concern. The methodology is specifically developed as a first screening tool, and can for instance be applied by emission licensing authorities, who are often confronted with the need to permit emissions for chemicals with limited or even no toxicity information available. When a chemical is considered to be of potential concern

based on structural similarity, a more in-depth analysis should be considered. Furthermore, the developed approach can help in selecting and prioritizing substances for follow-up within regulatory frameworks and thereby contributes to enhanced grip on chemicals of potential concern.

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Application of the RAIDAR Model to Aid Chemical Prioritization in Canada
J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology; J.M. Armitage, AES Armitage Environmental Sciences, Inc / Physical and Environmental Sciences; L. Toose, Liisa Toose Environmental Research; M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division
The traditional approach method (TAM) for chemical assessments is to categorize chemicals using various "bright-line" pass/fail criteria for Persistence, Bioaccumulation and Toxicity (PBT). However, new approach methods (NAMs) to screen and prioritize chemicals based on estimates of exposure and potential risk have recently emerged, an example of which is the Risk Assessment IDentification And Ranking (RAIDAR) modeling framework. RAIDAR combines chemical fate and transport at a regional scale with bioaccumulation in aquatic, terrestrial and agricultural food webs using toxicokinetic models thereby providing the opportunity for holistic exposure- and risk-based high-throughput and screening-level assessments. Here we conduct a case study to compare RAIDAR exposure- and risk-based metrics to PBT classifications for approximately 10,000 organic chemicals. Chemical property and toxicity data were compiled from various sources whereas emissions estimates were obtained primarily from estimated production volumes. Simulated exposures to a range of ecological receptors were quantitatively compared to toxicity data and expressed as chemical-specific Risk Assessment Factors (RAFs). The RAFs calculated for the 10,000 chemicals span approximately 18 orders of magnitude thus providing a single and relatively simple means for prioritizing the chemicals based on potential risks to a range of ecological receptors (i.e., aquatic and terrestrial species). Chemicals classified in this example as "PBT" and "not PBT" show an overlap of approximately 12 orders of magnitude when compared based on RAFs. In other words, many chemicals classified as "no concern" using PBT methods have risk estimates comparable to chemicals classified as "PBTs". This comparison indicates the potential for "false negatives" when considering PBT classifications and the potential for adverse effects to the environment. Conversely, many chemicals classified in this case example as ""PBT" have lower RAFs than many of the "non PBT" chemicals indicating the potential for "false positives" when seeking to identify chemicals with potential for adverse effects to the environment. The results also suggest that significant effort and resources can be spent prioritizing, testing and assessing chemicals that present a lower concern in the environment (i.e., are likely not true chemicals of concern), primarily due to a wide margin of exposure.

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Early identification of both chemical and microbial contaminants in the aquatic environment using scientific literature text mining
J. Hartmann, Dutch National Institute for Public Health and the Environment / Centre for Sustainability, Environment and Health; I. van Driezum, National Institute for Public Health and the Environment RIVM / Centre for Sustainability, Environment and Health (DMG); S. Wuijts, National Institute for Public Health and the Environment RIVM / Centre for Sustainability, Environment and Health; B. Venhuis, National Institute for Public Health and the Environment RIVM / Centre for Health Protection; A. de Roda Husman, National Institute for Public Health and the Environment RIVM / Centre for Zoonoses and Environmental Microbiology; J. van der Hoek, Delft University of Technology / Water Management
Contaminants in the aquatic environment pose a potential threat to ecosystems and to humans. Humans may be exposed to contaminants in water resources when used for drinking water production and recreation. Climatological, social and demographic changes as well as the increasing sensitivity of analytical techniques result in the augmented detection of contaminants. Thus, effective risk governance of contaminants in the aquatic environment is and will remain very important in order to protect human and environmental health. Recent research has shown that it generally takes about 15 years from the first onset of concern about a contaminant to the peak of concern and thus regulatory action or mitigation. The first onset of concern is often a scientific article. We hypothesize that one of the factors influencing the period of emergence of concern about contaminants is the fact that the first article about the contaminant is lost in the vast amount of publications. It is not until more articles are published about the specific contaminant that the signal is picked up by regulators. In this study, we therefore propose a methodology using text mining for identifying the first signal of the detection of a contaminant in the aquatic environment. The proposed methodology uses scientific literature text mining, which is the automatedtextual analysis of the combination of title and abstract. The methodology is R-based and consists of five steps: 1) a search in Scopus, 2) adding abstracts to the retrieved records using R, 3) pattern matching in R to semi-automate the primary study selection, 4) finish the primary study selection manually, and 5) check the national relevance of the identified signals. The search query used was a combination of four concepts that each included several synonyms. The four concepts of microbiological and chemical contaminants were *contaminant*, *detection*, *new*, and *aquatic environment*. The proposed methodology enables regulators to proactively assess relevant signals of emerging contaminants in the aquatic environment based on automated literature research. Next steps involve developing a targeted monitoring program to analyse water samples for the identified signals relevant for the environment and population in the Netherlands.

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Poster spotlight

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Micro(Nano)plastic Pollution: Tackling the Plastic Problem by Identifying Sources, Investigating Fate and Novel Approaches (IV)

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Simplifying Microplastics
M. Kooi, Wageningen University & Research / Aquatic Ecology and Water Quality Management Group; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management
Microplastics are perceived as complex. They include particles that are highly diverse in size, shape and density. Additionally, they consist of mixtures of polymers and chemical additives, and when exposed in the natural environment, absorption of chemical contaminants and the formation of biofilms further enhances their complexity. Many studies now struggle with the complex challenge that microplastics pose, and focus on small subsets of the microplastic matrix. Instead of focussing on the complexity and diversity of microplastics, we need to conceptually simplify microplastics. Microplastics can be seen as one entity, with a parameter space of probability distributions. A parameter space provides all combinations of values for all different plastic parameters, or properties, included in a study. Often, the individual properties are approximated using probability distributions, which describe how likely it is that a certain property values will occur. These probability distributions capture the diversity of the different properties, which for microplastics could include size, shape, density, colour, chemical composition, biofilm and attachment efficiency. For instance, microplastic particles vary in size between 100 nm and 5 mm according to most definitions. However, the majority of studies find that smaller particles are more abundant than large particles. Therefore, the particle size could be approximated using a for example a power-law probability distribution which results in a rapid decrease in particle number with increasing size. As another example, microplastic densities are often assigned discrete values for different polymers. However, in reality these densities overlap and form a more continuous distribution. Particles often contain mixtures of polymers, and once in the natural environment, biofouling, swelling and weathering could change the density of the particle. Using probability distributions to describe microplastic properties and studying the parameter space of these properties will greatly enhance our understanding of the complex microplastic matrix. The approach can be applied to exposure measurements, fate modelling, effect studies and ultimately risk assessments. When studying the risk that microplastics pose, instead of providing a yes/no answer for small subsets of microplastics, we could provide a probabilistic risk assessment for microplastic as a whole.

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PET Microplastic of Textile Origin - Size distribution, characterization and retention in a lab-scale Wastewater Treatment Plant
M. Lykaki, Technische Universitat Dresden / Department of Evironmental Sciences; Y. Zhang, Dresden University of Technology / UFT Centre for Environmental Research and Sustainable Technology Department Sustainable Chemistry; M. Markiewicz, Technische Universitaet Dresden; S. Stolte, Technische Universitat Dresden
Microplastics (MPs), particles < 5mm, are nowadays subject of intense interest and research due to the enormous annual release of plastics into ecosystems and their potential threat to human health. Among plastic debris, polyester fibers originated from synthetic textile shedding have been considered as an important source of contaminants. Over 40 million tons of polyester fibers alone are produced per year entering the environment through wastewater effluent. It has been estimated that a typical wash load of 5 kg polyester fabrics could release from 7×10^5 up to more than 6×10^6 microplastic fibers. The aim of this study is to understand the occurrence and fate of polyester fibers in dependency of their length. Therefore, fluorescent-labeled polyethylene terephthalate (PET) textiles have been washed in a household washing machine and the effluent passed through a filtration cascade including filters with mesh sizes between 1500 and 5 µm. Separated fibers were characterized through visual analysis with fluorescence microscopy and image analysis software. The different PET size fractions have further been investigated towards their retention in mechanical, chemical and

biological stages of Wastewater Treatment Plant (WWTP) on lab-scale. First results on the quantification of fluorescence-marked PET fibres from washed polyester textiles demonstrate that the highest release of MP was recorded for two size fractions, 500-150 µm and 150-50 µm (fractions smaller than 5 µm were not quantified). Laboratory tests simulating WWTP processes suggest that MP fibres could be efficiently removed (>80%) from complex matrices such as activated sludge. Furthermore, it was demonstrated the use of flocculants such as $Al_2(SO_4)_3$ facilitated the aggregation and precipitation of fibres. This is the first study focusing exclusively on the performance of polyethylene terephthalate (PET) fibers, one of the most common materials used in the apparel industry and demonstrating that the behavior of textile-origin microplastics in wastewater treatments may be size and density dependent. The results could provide a better understanding on the impact of MPs in the environment and suggesting innovative techniques for possible future monitoring purposes.

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Nanoplastic in a porous media: improbable transportation or realistic deposition?

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Nanoplastics (NPs), defined as colloids that originate from the unintentional degradation of plastic, have recently been detected in the North Atlantic Ocean. As a colloid in aqueous systems, NPs present a continuum in sizes ranging from 1nm to 1μm, associated to asymmetrical shapes and Brownian motion [1]. These particles could pose specific ecological and health risks. In the life-cycle of a plastic debris, the terrestrial compartment is the main source and water is the main vector. So it appears crucial to describe NP transport in soils. Up to now, the large majority of published results concerning the ecotoxicological impact and environmental behavior of NPs used engineered plastic nanomaterials (polystyrene latex spheres) whose properties are significantly different from those of NPs (ie: monodisperse and spherical) [1]. In this study, we tackle such approach by describing the fate of different types of nano-sized plastics in a porous media, in conditions that are unfavorable to attachment (ie: repulsion). Size standard polystyrene (PS-std) latex spheres and an environmentally-relevant NP model (PS-m) were used. The parameters discriminating each of these particles are their average hydrodynamic diameters (200 to 500nm), surface properties (presence or absence of carboxylate functional groups) and shape (spherical or asymmetrical), mimicking more or less closely NPs. Their transport and deposition was evaluated using colloid filtration theory, DLVO theory and hydrodynamic modeling. Our results show that PS-m are more likely to be trapped in the porous media compared to the standards used in "nanoplastics" environmental investigation (PS-std). PS-m also undergo changes in size, unlike PS-std. The presence of geochemical heterogeneities in the porous media may increase adsorption of PS-m and carboxylated PS-std. Deposition was mostly reversible, suggesting that the particles may be remobilized. This study suggests to reconsider the existing data on the environmental behavior of NPs in porous media since engineered plastic nanomaterials have limited to no environmental relevance. 1. Gigault J, Halle A ter, Baudrimont M, et al (2018) Current opinion: What is a nanoplastic? Environ Pollut 235:1030–1034. https://doi.org/10.1016/j.envpol.2018.01.024

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Occurrence of microplastics in commercial table salts and its implication to environmental fate and human exposure

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Salt intake is of particular concern to human exposure to microplastics (MPs). Previous studies which have identified MPs in commercial table salts including sea salt, rock salt, lake salt, and well salt showed a large variaiton in abundance (0 – 40,000 MPs/kg) and different composition profiles of MPs. Significant differences among studies in MP analysis methods makes it difficult to compare their results directly, to address the origin of MPs in salts, or to estimate human exposure. A reliable picture about geogrpahical distributio of MP pollution in global scale is available just when harmonized and consistetn method is applied. Recently, we determined the abundance and distribution characteristics of MPs in 39 table salt brands which are sold in supermakerts of 17 countries, including 28 sea salts from 16 countries, 9 rock salts from 8 countries, and 2 lake salts from two countries. FTIR analysis confirmed that MPs in each salt was not associated with its packing material. In entire salt samples, MP particles were detected with a range of 0 – 13,629 MPs/kg: 675±2560 MPs/kg in sea salt, 38±55 MPs/kg in rock salts, and 245±307 MPs/kg in lake salts. The distributions of polymers, shapes, and colors of MPs varied among salt types. MPs

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Co-gasification of microplastics and biomass: syngas quality index

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Microplastics have been considered one of the most concerning environmental issues as reported in recent reviews [1-3]. They originate from plastic products which are utilized and disposed of on a daily basis, supporting bioaccumulation effects that reach higher levels in the trophic chain [4-6]. Gasification embodies an interesting disposal option for plastic waste streams, granting not only environmental advantages but also the achievement of syngas, a commercial gas composed of CO, CO_2, H_2 and light hydrocarbons which might be utilized to produce marketable assets [7]. These include electricity, chemical products and even hydrogen or similar fuels for the transport sector [8, 9], which could even be used as an alternative to replace fossil fuels [10]. Gasification of plastics has been studied for the last decades as an attempt to substitute current waste processing techniques by more sustainable options, simultaneously contributing to this bolder aim [11, 12]. Several studies report the gasification of plastic debris as a possible technique to reduce their presence on the environment [13-16], some even suggesting the conversion of microplastics [17, 18]. Actually, gasification might portray some difficulties, i.e. problems in the feeding step and the formation of contaminants if plastic materials are treated alone. Yet, these may be attenuated if biomass is used as co-fuel [13, 15, 19]. This work aimed to evaluate the syngas produced from the gasification of different mixtures of PET (polyethylene terephthalate) microplastics and biomass, namely its quality and possible utilizations. This was attained through numerical runs with the software ASPEN Plus, which enabled the optimization of the experimental conditions. This strategy supports the dual benefit of Waste-to-Energy techniques, reducing the amount of plastics in the environment as well as sustainably generating commodity assets that currently rely on fossil fuels. Therefore, mixtures of microplastics and biomass within a range of 0% PET to 90% PET were assessed and some quality indicators were calculated. Higher CO and H_2 yields were seen for higher PET fractions, which enabled dedicated end-use applications for the achieved syngas.

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Are "bioplastics" a safe alternative to conventional plastics? In vitro toxicity of extracts from plastic products

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It is well established that chemicals intentionally or non-intentionally present in plastics, that is, plastic additives and side products, migrate from consumer products, thus representing a source of exposure to wildlife and humans. Previous studies have demonstrated that chemicals in plastic food contact materials (FCMs) induce toxicity *in vitro* and *in vivo*. Bio-based and biodegradable plastics are considered as a sustainable alternative for their petroleum-based counterparts. However, data is missing whether they also represent a safer alternative regarding their chemicals' toxicity. Thus, our study aims at comparing the toxicological and chemical profiles of a range of everyday FCMs and their precursors made of bio-based and/or biodegradable polymers. We extracted 44 products currently available on the German market made of polyethylene (Bio-PE) and polyethylene terephthalate (Bio-PET) derived from renewable resources, polybutylene adipate terephthalate (PBAT) and polybutylene succinate (PBS) derived from fossil fuels as well as polylactic acid (PLA), polyhydroxyalkanoates (PHA), cellulose, bamboo- and starch-blends, which are both biobased and biodegradable. We analysed the *in vitro* toxicity regarding unspecific (baseline toxicity, oxidative stress response) and endocrine endpoints (estrogenicitiy and antiandrogenicity). We characterise the chemical composition by non-target high resolution mass spectrometry (LC and GC-QTOF-MS). Out of the 26 products analysed so far, all contained chemicals triggering baseline toxicity. Here, we observed the highest toxicity for cellulose-based products. While seven extracts activated an oxidative stress response and five inhibited the androgen receptor, no sample contained chemicals activating the estrogen receptor. Overall, at least one product of each material induced at least one endpoint in addition to baseline toxicity. Exceptions are the PHA and bamboo-based products for which we have analysed one product only. Our findings indicate that extracts of bio-based and biodegradable plastic products can induce a range of toxicological endpoints and are not necessarily a less toxic alternative to petrol-based materials. The ongoing chemical analysis will reveal their chemical composition and clarify whether the "bio-materials" contain the same substances as conventional plastics.

Fate and Effects of Metals: Advances in Metals Risk Assessment and Regulatory Guidance (II)

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Risk management of arsenic inherited from mining activities and World War I in the agricultural soils of Europe

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Finland
Arsenic is a toxic and carcinogenic semi-metal. Greater than average As concentrations may occur naturally in bedrock and soil, but major risks in agricultural soil are linked with pollution. European geochemical mapping programmes have revealed that in central and southern Europe, As concentrations are higher on average than in northern Europe. In 2017, we started the AgriAs project to investigate the speciation and transformation of As in soil, its uptake by plants and impact on vegetation. The goal has been to evaluate the risks through agriculture and provide tools for risk management. Recommendations have been compiled in close cooperation with authorities and stakeholders. Our study sites, Verdun in France and Freiberg in Germany, exhibit As contamination in agricultural soils. Over one billion shells and projectiles were fired in Europe during World War I. After the war, over three Mt of abandoned and unexploded ammunition had to be destroyed in northeast France for safety reasons, among them a huge amount of As-bearing chemical agents. At many destruction sites, topsoil contamination is still severe. The results of the AgriAs sampling campaign in Verdun showed As concentrations from 15 mg/kg in the reference zone to 775 mg/kg in a more contaminated location. Arsenic is phytoavailable and toxic to plants. After 800 years of mining in the Ore Mountain region in Saxony (Germany), 288 km^2 of agricultural soils showvalues exceeding the German action value of 50 mg/kg As. The centre of historical mining in Freiberg mainly shows concentrations above 320 mg/kg. Alluvial soils in the floodplains along rivers in this region have As concentrations from 80 to 320 mg/kg due to drainage water from mining tunnels and discharged process water and sediments from ore processing. Risk and recommendations have been communicated by the German authorities for As-rich soils used for agriculture and gardening as well as residential areas. The AgriAs project will develop more general recommendations for the risk management of arsenic in European agroecosystems. These recommendations will include an overview of sources and pathways of As and a summary of European legislation with national guideline values. Recommendations will be developed for site characterization, risk assessment and risk management including good agricultural practices and water management. Recommendations for further research and policy development will be finally summarized.

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Copper toxicity to fish in natural Amazonian water: The influence of dissolved organic matter
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Dissolved organic matter (DOM) is known to alter metal bioavailability to aquatic organisms, by effectively binding them in natural waters. For some metals (e.g. Cu), this effect is so considerable that it is incorporated into bioavailability-based models (e.g. Biotic Ligand Model, BLM), used to assess the environmental risk of metals in various legislations (e.g. EU Water Framework Directive). However, most of the research on this topic is on temperate regions, while tropical biomes and fish species have been far less studied. This paucity of data contributes to the great gap in developing site-specific water quality criteria in these ecologically important regions. Today, there is a need to better characterize how bioavailability concepts apply to these unique environments, notably with respects to metal-DOM-fish interactions. In the present study, we investigated how the DOM of the Rio Negro (RN), a major tributary of the Amazon river, affects acute Cu toxicity to a local fish: the cardinal tetra (*Paracheirodon axelrodi*). There is evidence that this RN DOM interacts with fish gills, resulting in a positive effect on fish Na homeostasis, the main target of acute Cu toxicity in freshwater animals. In a previous study, we suggested that this physiological protection may add to the extent of protection offered by Cu-DOM complexation, as predicted by the BLM. The present study aimed at further investigating this potential dual role of the RN DOM against Cu-induced Na imbalances in fish. We acclimated *P. axelrodi* to freshly collected RN water (10 mg L^{-1} DOC) and to a DOM-diluted water (1.4 mg L^{-1} DOC) with equal pH (~ 5.7) and major cations concentration (≤ 40 µM). In both waters, we first measured 3-h Cu-gill uptake within Cu concentration ranges of acute toxicity, using the stable isotope Cu^{65}. We assessed whether gill accumulation followed the determined free Cu^{2+} concentration in the water, in accordance with the BLM. Second, we related these Cu-gill bindings with measured unidirectional Na^+ fluxes at the gills. For these two experiments, various DOM pre-acclimation times of 0, 1 and 5 days were tested. Initial findings suggest that $[Cu^{2+}]$ in the water is a relatively good predictor of Cu-gill binding, which in turn appears to be a good indicator of Na^+ ionoregulatory disturbance. These preliminary results indicate that protection by DOM mainly occurs from Cu complexation under the tested conditions, in support with a key concept of the BLM.

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Updating the chronic Copper bioavailability models
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The current procedure for deriving bioavailability-based Predicted No-Effect Concentrations (PNECs) for Cu as outlined in the Cu voluntary risk assessment report (Cu-VRAR, 2008) is based on bioavailability-normalisation of chronic toxicity data with chronic Cu bioavailability models for three trophic levels: a *Daphnia magna* Biotic Ligand Model (BLM), an algae generalized Bioavailability Model (gBAM) and a fish BLM. Overall, this work aimed at refining the copper bioavailability models by reviewing and incorporating recent advances in speciation modelling and copper toxicity modelling that have emerged over the past 10 years. Over this period, optimizations of the Cu bioavailability models for fish and *D. magna* have been proposed through the use of generalized bioavailability models. These updated bioavailability models account for other factors that determine the effect of pH on free metal toxicity besides the competitive effect of H^+ at the biotic ligand site, and as such may more accurately predict the effect of pH on Cu^{2+} toxicity than the BLM. In the current Cu-VRAR (2008), chronic bioavailability models are used in combination with WHAM V as geochemical speciation program. However, a more user-friendly version of the Windermere Humic Aqueous Model (WHAM VII) has become available meanwhile. Yet, to be able to update the entire chronic Cu bioavailability normalization procedure to a WHAM VII/gBAM-based approach, a validation of the algae and *D. magna* gBAMs in WHAM VII is still needed. Therefore, we evaluated in the present study whether chronic Cu gBAMs for algae and *D. magna* originally developed in combination with WHAM V can be updated to WHAM VII without compromising on the predictive capacities of the original models. To this extent, we first reparametrized the pH slopes (S_{pH}) of the existing algae and *Daphnia magna* gBAMs based on WHAM VII-calculated free Cu^{2+} activity. Next, it was shown that the newly parametrized WHAM VII-gBAM can be used to predict chronic dissolved Cu toxicity to several model- and non-model-species within 2-fold error. Overall, our analysis showed that the updated bioavailability models for chronic toxicity of copper, based on the gBAM model structure and incorporating a more recent version of the speciation tool (WHAM VII), can be used to accurately predict chronic toxicity to both model- and non-model species. These updated models may be useful for the purposes of environmental hazard and risk assessment of copper.

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Ecotoxicity of lanthanides to Daphnia magna
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Lanthanides (LNs) are a group of fifteen elements with increasing economical importance in sectors such as clean energy, military industry and agronomy. However, the available information on their ecotoxicity still remains quite limited and only a handful of studies have examined the biological effects of the whole series of LNs. Understanding the ecotoxicity of the entire LN series is important because it is not yet clear if biological effects follow a regular pattern along the series; which may simplify the hazard and risk assessment for this group of elements in the future. In this contribution, the acute toxicity of the entire series of LNs to *Daphnia magna* was evaluated according to the ISO standard 6341 along with a study of the chemical behaviour of LNs in the ecotoxicological exposure medium. ECs50 ranged from 6.8 to 30.5 mg/L (0.075–0.18 mM) after 24h and from 4.3 to 15.7 mg/L (0.04–0.1 mM) after 48h and did not show any clear trend along the LNs series. Total LNs concentrations measured at t=0 agreed with nominal values to ± 20% with the exception of Nd (156%) and Dy (60%). Filterable concentrations decreased by 45–95% after 24 h and by 50–98% after 48 h. Total concentrations after 48 hours remained within 25% of initial values for Nd, Tb, Ho, Tm, Sm and Lu, but decreased by 50% or more for the other lanthanides. Thermodynamic model calculations (VisualMinteq, ver 3.1) indicate that precipitation of LNs carbonates can occur at the elemental concentrations added into the test vessels; in agreement with the experimentally measured decrease of (filterable) concentrations in the test medium. In such situations, ECs50 are better expressed using time-weighted mean (TWM) values that allow to calculate the average concentration to which organisms are exposed over the entire test duration. Using the TWM approach actually reduces the risks of underestimating the ecotoxicity of lanthanides to *D. magna* and hence the possibility of errors in hazard and risk assessement. ECs50 can also be expressed based on the modelled concentrations of the free ionic species (Ln^{3+}) of each lanthanide to facilitate intercomparability between tests and laboratory-to-field extrapolation of standard ecotoxicological results. Care must however be taken in interpreting combinations of model results (valid for thermodynamic equilibrium conditions) and dynamic situations in laboratory and field settings.

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QICAR as a tool to address the lack of toxicological data for technology-critical metals
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The development of new technologies requires the use of metals (Au, In, Ge, platinum group elements) for which impacts on aquatic ecosystems are poorly known. Quantitative structure-activity relationships (QSAR) have been used for many years now to address this issue for newly formulated organic molecules. In the present study, we developed Quantitative Ion Character Activity Relationships (QICAR) to predict the toxicity of technology-critical metals (TCM) towards aquatic organisms based on intrinsic metal characteristics. Twenty-three metal characteristics (e.g.: molecular weight, atomic radius, electronegativity, …) were evaluated as predictors of metal toxicity. Modelled organism responses were the measured acute EC_{50} values of 12 *data-rich* metals for algae, daphnids and fish. Models were constructed with and without species-distinction. Both total dissolved metal concentrations and the free metal ion activities were used to build the QICAR. Prior to model development, principal component analyses were used to highlight autocorrelations among metal characteristics and to reduce the number of entry variables. Simple and multiple linear regressions (stepwise approach) were then constructed and examined for their goodness of fit statistics as well as verified for their linear regression assumptions. Finally, QICAR were tested by comparing the measured and predicted EC_{50} values of the TCM. For QICAR based on the free metal ion activities, linear free energy relationships were additionally built to estimate the binding constants of the data-poor metals with HO^-, CO_3^{2-}, EDTA and Cl^- when these values were lacking. Among the 15 'best' obtained QICARs (adjusted $r^2 > 0.8$), 12 were simple linear regressions with X_m^2r as predictor. This composite parameter (X_m =metal electronegativity; r = metal ionic radius) is a proxy for the metal's ability to form covalent bonds with ligands present in the exposure media and in the test organisms. The toxicity of the TCM was found to be best predicted by the QICAR models based on the total dissolved metal concentration rather than those based on the free metal ion activities. This unexpected result might reflect the fact that the tested *data-poor* metals form poly(hydroxo)-complexes in the exposure media, leaving only very low free metal ion concentrations ($< 10^{-15}$ M). Future experimenters might want to focus on the study of the interactions between such complexes and the test organisms.

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Use of AF on HC5 for data rich metals, revisiting the evidence !
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Statistical extrapolation in line with the provisions of the WFD-EQS and REACH guidance, namely the species sensitivity distribution method (SSD), can be used for the derivation of EQSs for data rich metals such as Cu or Zn. The median estimate of the HC_5 is proposed as the basis of the EQS/PNEC. According to the requirements set out in the regulation, an SSD can only be constructed when data are plentiful but there may still be some residual uncertainty that needs to be accounted for in the final EQS/PNEC. For this reason, the HC_5 is divided by an additional assessment factor (AF): EQS (or PNEC) = HC_5/AF. The choice of the AF applied to the HC_5 is dependent on the remaining uncertainty associated with the assessment. According to the current approach, metals (or substances) with similar HC_5 and residual uncertainties need to be protected at different levels depending on the NOEC ranges, ie. a metal with a narrow range of NOEC request a higher protection level than a metal with a broader range of NOEC values. However from a scientific perspective, similar protection levels are deemed to apply for substances with similar HC_5 and residual uncertainties. Moreover, the current approach does not consider the sensitivity (ie. slope) of the SSD as it derives similar EQS/PNEC for SSD with very different slopes. Therefore, an alternative approach is proposed to derive safe threshold concentrations for data rich metals/substances. In this new approach it is proposed to apply the AF directly on the protection level or PAF (Potentially Affected Fraction) value (ie. 5% according to the regulation) rather than on the concentration derived from the protection level, ie. the HC_5. To assess how this newly proposed approach would affect the setting of EQS/PNEC values, different real world examples have been assessed. As an example, the SSD of Zn have been re-evaluated for EQS/PNEC setting. In the current REACH/WFD methodology, a HC_5 of 0.0156 mg/L has been calculated. Considering an additional AF of 2, this is resulting in a EQS/PNEC of 0.0078 mg/L (Zn RAR, cfr. EC, 2004). However, applying the newly developed approach on the Zn SSD would result in a EQS/PNEC value of 0.0117 mg/L. Similar findings were observed for Ag, where a EQS/PNEC of 40 ng/L is reported in the REACH dossier, while a EQS/PNEC of 60 ng/L is calculated from the newly developed approach.

Modelling and Monitoring of Pesticides Fate and Exposure in a Regulatory Context (I)

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Updates on Surface Water Model Development in China for Higher Tier Environmental Risk Assessment
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Here we update the findings from a science collaboration project between Nanjing Institute of Environmental Science (NIES), China and Syngenta. The aim of the project is to support the development and evaluation of dry-land surface water modelling in China for higher tier environmental risk assessment for plant protection product registrations. Local dry-land field data including soil, weather, hydrological data, and agronomic practice information have been collected by NIES for eight sites representative of Chinese agricultural areas. All data were processed and parameterised in PRAESS taking into consideration a vast database of scientific literature and in depth knowledge of European and American environmental risk assessment approaches. Head-to-head comparison between PRAESS and EU FOCUS surface water model, has given some confidence in the model performance. Future recommendations for higher tier risk assessments in China are bridged with a European higher tier case study. In this case study, exposure profiles derived from FOCUS surface water modelling outputs were combined with toxicokinetic and toxicodynamic models as a weight of evidence to demonstrate safe use and maintain registration in Europe. This science collaboration work has laid a solid foundation for a Tier II surface water model acceptance for refining product regulatory assessments in China. The European case study provides a useful and forward-looking framework to further develop refinement methodologies for ecological risk characterisation in China; thus, appropriate protection goal and definition settings are essential for ecological risk assessment.

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A critical reflection on the use of Briggs´ equation in pesticides regulation
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Briggs *et al.* (1982) examined the relation between TSCF and the octan-1-ol water distribution coefficient logKow based on experimental uptake data for 18 substances. The authors found a bell shaped correlation and derived a respective equation. This equation was later applied as standard procedure to obtain TSCF values for groundwater modelling in ppp regulation. Using the substance specific logKow, TSCF values can be calculated individually. A general TSCF value of 0.5 was derived as the approximate average from this study and was established as a default TSCF for systemic, non-ionisable substances in groundwater modelling in ppp regulation. For non-systemic and ionisable substances a default of 0 was recommended (FOCUS, 2000, p. 94; FOCUS, 2002, p. 38). Since the publication of the original paper in 1982, more data on plant uptake of chemical substances has become available. The correlation of the uptake to one single factor, the logKow, has been queried by several authors, e.g. due to the lacking precision of single-parameter relationships and the limitation of these relationships to a wide variety of contaminants and plants species (Bagheri *et al.*, 2019). Trapp and Pussemier (1991) measured the uptake of carbamates in bean plants and concluded that the Briggs equation does not fit the data. The authors discussed the possibility that the difference might have been caused by different lipid contents of the bean plants and the barley plants used by Briggs. Dettenmaier *et al.* (2009) analyzed 126 TSCF values of 115 substances from various studies and found a high variability. The relation between logKow and TSCF, as reported by Briggs (1982), was not confirmed. An even bigger dataset was analyzed by Doucette *et al.* (2018). The authors separated neutral from ionisable compounds and linked them to the empirical relationships described by Briggs (1982) and Dettenmaier *et al.* (2009). Compared to the dataset of Dettenmaier *et al.* (2009), the scatter is reduced. However, a clear relationship between the TSCF and the logKow has also not been established. In our presentation we review and analyze available data and demonstrate why we conclude that neither the Briggs equation nor the default of 0.5 are adequate and conservative enough to be used in groundwater simulation models. Consequently, there is an urge to reject the temporary regulatory approach to use of TSCF values based on the Briggs equation.

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DEVELOPMENT OF A OECD TEST GUIDELINE TO DETERMINE PLANT ROOT UPTAKE FOR REGULATORY ENVIRONMENTAL FATE MODELLING
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A novel OECD study design is currently under evaluation by a group of experts from academic, industry and regulatory, which can be used to quantify uptake of chemicals via the root system of plants. Ten laboratory organizations with different levels of experience with uptake testing will participate in a round robin test and study uptake of [^{14}C]-1,2,4-triazole by oilseed rape and wheat plants. The

aim is to examine the reproducibility of the results obtained with the proposed study design and their reliability for regulatory purposes. The study design has already been tested investigating uptake of different compounds (molecular weight: 114 to 563 g/mol; log Kow: -1.5 to 2) by wheat, tomato and potato plants. Furthermore, the design may not be appropriate for volatile compounds, highly phytotoxic compounds, or quickly degrading compounds. At the moment the study design has been tested using wheat, potato, tomato and oil seed rape plants, but is not limited to these. To explore the possible impact of different soil pH´s, the experiments will be conducted at two pH values: pH 5.5 and pH 7.0. The The plant uptake factor (PUF) and the transpiration stream concentration factor (TSCF) will be calculated for the three different incubation periods ending after t = 2, 4 and 6 days according to the following equations that consider the concentration development over the course of the incubation. The envisaged study design was explicitly developed to address the need identified by EFSA and was based on suggestions from the EUregPUF workshop (York, 2013) where participants from academia, authorities and industry met to establish an up to date understanding of plant uptake science. Lessons learned from the testing and continuous exchange with academia and authorities facilitated optimisation of the study design. Critical issues that were already identified within the OECD process (e.g. a possible concentration dependence of the uptake or the duration of the experimental phase) will be investigated and discussed. The current version of the study design is evaluated during the course of the OECD process to answer the question if the test design can be considered appropriate to produce reliable data on plant uptake to be used as input for refined exposure modelling. An explicit guidance, on how to integrate the requested study design into the regulatory process is aimed to be developed based on the outcome of the final OECD test guideline.

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Model comparison of the effects of plant uptake on the environmental fate of three anonymised pesticide compounds
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The potential hazards of plant protection products (PPPs) require regulation via pesticide registration which requires a simulation of their environmental fate. Within the EU regulatory framework, the working group FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe) proposed the main regulatory models to be PEARL, PELMO and MACRO, with PRZM being used sparingly. A lack of consistency and reproducibility in experimental laboratory plant uptake factor (PUF) tests has led to low confidence in the ability to determine a consistent PUF value. Regulatory advice now states that plant uptake within pesticide fate models should be set to PUF = 0 unless the compound is known to be systematic, at which a PUF = 0.5 value is supplied. Despite this, only a small amount of work has been conducted with regards to the effect of plant uptake on Predicted Environmental Concentration in Groundwater (PEC_{GW}). This study addresses how the outputs of different pesticide fate models depend on changes in PUF, and what implications this could have for environmental fate modelling going forward. PEARL and PELMO were selected for this study given their varied approaches to modelling the soil water balance and dynamics. A systematic approach was taken to scenario selection, with Eurostat databases providing crop production and pesticide sales statistics, which were used to rank the locations and select those appearing highest for both categories. Compounds were selected to represent a range of mobility and persistence values, therefore, a range of soil adsorption coefficient (K_{OM}), half-life (DT50) and PUF values were used, with all other parameters being kept constant. Both models were run for 26 years, with a daily time step, and a 6-year warm-up period included. Comparisons of the outputs show that both models behave very differently across locations; this appears to be particularly true for sandy soil profiles with high irrigation inputs over the summer. The effect of plant uptake (modelled from 0 to 1) on PEC_{GW} values in PEARL has a range of 5%-38%. Results from this study show that agreement between both models is highly dependent on physical soil properties and this requires further study. *Acknowledgements* – BBSRC for funding this project and Syngenta for being a CASE partner.

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Introducing PERSAM v3.0: a tool for predicting environmental concentrations in soil
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European pesticide authorisation requires risk assessment procedures using standardised exposure scenarios developed by the European Food Safety Authority (EFSA). Recently EFSA produced a revised Guidance Document providing guidance for the exposure assessment of soil organisms to plant protection products (PPPs) and their transformation products in accordance with Regulation (EC) No. 1107/20091 of the European Parliament and the Council. Guidance is provided for all types of concentrations that are potentially needed for assessing ecotoxicological effects, i.e. the concentration in total soil and the concentration in pore water, both averaged over various depths and time windows. The guidance considers both permanent crops and annual crops. The goal of the exposure assessment is the 90th percentile of the exposure concentration in the intended area of use in each of the three regulatory geographical zones (North, Centre, South) or in a selected EU country. The recommended exposure assessment procedure consists of four tiers where all tiers aim to assess the same exposure assessment goal. In higher tiers of the exposure assessment, crop interception and subsequent dissipation at the crop canopy may be included. Given the complexity of the calculations and parameter selection in the different tiers, efficient use in the regulatory process requires the development of software tools. PERSAM was developed to assist the user in performing calculations using the analytic model, as described in the guidance document and the latest release is developed in line with the revised guidance. The software is able to calculate (i) Tier-1 predicted environmental concentrations (PECs), (ii) Tier-2 95th-percentile PECs and to (iii) select the grid cell (including the scenario properties) corresponding to the 95th-percentile PEC as needed for the scenario development at Tier 3A. PERSAM can also generate a so-called transfer file that can be read by the numerical models described in the Guidance Document. PERSAM generates reports intended for regulatory submissions. The concepts of the calculation procedure will be discussed and the software will be demonstrated with particular attention for the new features compared to previous versions of the software.

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Metaldehyde dynamics in a small agricultural catchment
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Pesticides make important contributions to modern agriculture but losses from land to water can present problems for environmental management, particularly in catchments where surface waters are abstracted for drinking water. In the UK, one of the most problematic pesticides facing water companies is metaldehyde – the active substance commonly used in slug pellets, which unusually difficult to remove via conventional drinking water treatment. A better understanding of metaldehyde dynamics is often hampered by low sampling frequencies. Here, we present a study of metaldehyde dynamics in a small (3.9 km^2) surface water catchment in Cambridgeshire, UK using a combination of high frequency in-stream monitoring and numerical modelling. Discharge was measured continuously at a Venturi flume. Water samples were collected every eight hours and analysed for metaldehyde using direct injection LC-MS. A simple conceptual hydrological and pesticide transport model was employed based on continuous root zone water balance calculations. Metaldehyde losses are calculated based on the displacement of a limited fraction of the soil pore water during rainfall events occurring after application. Metaldehyde pellets were applied in the second week of August 2014 to an unknown fraction of the catchment. Measured concentrations in stream water increased markedly in early storm events (to about 9 mg L^{-1}). Thereafter, concentrations “rachet down” in successive events - responding to rainfall but not to the same levels (i.e. reflecting an effective exhaustion in soil supply as a consequence of in-field degradation and dissipation). Insights derived from the application of the model to the study catchment will be presented.

Fish Model Species in Human and Environmental Toxicology (I)

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Sex Related Gene Expression Patterns in Zebrafish at Different Ages
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Fish embryos have been used to determine acute toxicity in fish embryo acute toxicity test (FET) and to measure effects of substances in the early life stage toxicity test (ELS). In contrast to rodents, one disadvantage to the application of fish toxicity tests is that gender-based selection of individuals cannot be done before or after the test. Consequently, the data obtained contains a mixture of male and female effects. This is important to note as several zebrafish studies showed gender differences. In contrast to the otherwise well-established gene annotation, *Danio rerio* still lack any clearly-defined sex-determining genetic loci. Recent studies suggest a polygenetic sex determination, where the genes influencing the sex-determination are distributed throughout the whole genome. In this study gene expression patterns of nine genes contributing to the sex determination and gonad differentiation, namely *sox9a, sox9b, dmrt1, cyp19a1a*, *cyp19a1b, cyp17a1, nr0b1, igf3* and *vtg1,* were determined by qPCR in zebrafish embryos which had been treated with beta-oestradiol or testosterone and also in different aged zebrafish. Although some of the genes showed sex-specific expression (*cyp17a1, vtg1* and *cyp19a1a),* no defined expression pattern could be observed to distinguish sex in neither larval stage nor juvenile zebrafish.

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Map and Model - how to make zebrafish toxicogenomics more predictable
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UFZ / Department Bioanalytical Ecotoxicology; J. Krüger, Helmholtz Center for Environmental Research - UFZ; K. Reiche, Fraunhofer Institute for Cell Therapy and Immunology / Bioinformatics Research Unit Department of Diagnostics; W. Busch, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology
Toxicogenomic methods using model organisms like the zebrafish are discussed to provide improved assessment of chemical related hazards or environmental health. At the same time, toxicogenomic data bear the challenge of comparing and interpreting highly complex molecular profiles, even when considering only one exposure condition. The integration of time or concentration dependent measurements even adds another layer of complexity. In order to improve comparability between toxicogenomic experiments we developed an experimental and bioinformatic pipeline to obtain dynamic toxicogenomic fingerprints of chemicals. We exposed zebrafish embryos (ZFE) to a range of five different concentrations of the substances diuron, diclofenac, and naproxen, and measured the transcriptome at five time points. The measured fingerprints were projected to an universal coordinate system (zebrafish toxicogenomic universe), based on a self-organizing map of compiled toxicogenomic data from public databases. Subsequently, we applied regression modeling to get a quantitative description of the time and concentration dependent responses. The resulting toxicogenomic fingerprints allow three dimensional inference on the concentration as well as the time scale to conditions not measured. Furthermore, the toxicogenomic universe allows to integrate extensive toxicogenomic information. Taken together this novel approach facilitates comparison between different fingerprints (and different studies) as well as between the responses of different clusters in one fingerprint. For our investigated substances we could identify and quantitatively describe the dynamics of key events in response to the exposures (related to e.g. developmental delay, stress response and cox inhibition) and discriminate between stage dependent and exposure dependent events.

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Toxicity and metabolomics exposure studies of zebrafish to PFASs and alternatives - chemical alternatives assessment approach
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Perfluorinated compounds (PFCs) are widely used as surfactants in industrial and consumer products, commonly as repellants in textiles, fire retardants and food packaging. However, many PFCs show to be persistent in the environment. In this study, chronic and acute exposures of PFOA, PFOS and its alternatives PFHxS, PFBS, GenX, D4, D5 and TMS were assessed. In this study we performed a chemical alternatives assessment strategy to compare the affected metabolomic pathways between PFOA, PFOS and the alternatives to assess more human and environmentally friendly compounds. Chronic and acute exposures of PFOA and PFOs and its alternatives PFHxS, PFBS, siloxanes (D4, D5 and TMS), and GenX were assessed using embryo (*Danio rerio*) to study the mechanism of toxicity and study if PFHxS, PFBS, and siloxanes are feasable alternatives. Zebrafish embryos were exposed to PFOA, PFHxS, PFBS, PFOS, GenX, D4, D5 and TMS, and the swimming activity and locomotor activity were recorded. No significant effects in mortality, developmental abnormalities, and swimming activity were observed in the exposed groups of all compounds. qPCR and metabolomic analysis showed that transcription of the DNA damage gene was significantly up-regulated for PFOA, PFHxS, PFOS and Genx. Interestingly, the lipid metabolism gene was up-regulated for PFOS and GenX. In the acute exposure, all compounds significantly affected phosphatidylcholines (PC) lipids, and PFOA, PFHxS and PFBS affected phosphatidylethanolamines (PE). In the chronic exposure, PC and PE were also affected, however, in contrast to the acute exposure, chronic exposure affected the triacylglycerols (TAG) and sphingomyelins (SM). TAG function as energy reserve and their metabolism plays a key role in cellular energy balance, lipid homeostasis, growth, and maintenance. The results showed that exposure to PFCs and their alternatives induce changes in lipid metabolism, mainly the glycerophospholipids pathway. The zebrafish embryo test showed to be a suitable assay for testing the toxicity and mode of action of environmental toxicants. Based on the results of this study, alterations in lipid metabolism (glycerophospholipids) is a metabolic pathway affected by both PFASs and their alternatives. The alternatives D4, D5 and TMS appears to have a lower impact on metabolic pathways (e.g. lipids) than PFOA.

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Differences in POPs, transcriptome and proteome of wild and hatchery-reared Baltic Salmons
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Despite conservation, the marine survival rates of both wild and hatchery-reared Baltic salmon (*Salmo salar*) are declining and lower survival is especially a problem with reared fish. Although some studies on the molecular level differences in wild and aquacultured Atlantic salmon has been done, there isn’t many on the Baltic populations. Salmon samples were collected in the years 2007 and 2008 from Baltic main basin (BMB), Bothnian Sea (BS) and Gulf of Finland (GoF). Liver from wild and reared salmon from each area were used for transcriptomic, proteomic and POPs analyses. POPs levels between wild and reared salmons showed a statistical difference only in the GoF for dioxin-like and non-dioxin-like PCBs. Comparison among areas showed that dioxin-like PCBs were significantly higher in GoF than in BMB or BS, while non-dioxin like PCBs were higher in BS than in GoF or BMB. Chlordane levels were higher in BS than in GoF and BMB, while o,p’-DDT and p,p’-DDT showed an opposite trend. BS had the highest number of DEG when comparing wild and reared salmons, followed by GoF and BMB. For DEP the order was GoF>BS>BMB. When comparing different areas within wild fish, BMB vs GoF had the most DEG, followed by BMB vs BS and BS vs GoF while for DEP the order was BMB vs BS>BS vs GoF>BMB vs GoF. For reared salmon, BMB vs GoF had the highest number of DEG and DEP, followed by BS vs GoF and BMB vs BS. In the comparison of wild vs reared salmon transcrptome, lipid metabolism pathways were enriched in BS and GoF, no enrichements were found in BMB. In wild salmons from different areas, main pathways were cell growth and death and signal transduction in BMB vs BS or GoF, while in BS vs GoF, lipid metabolism was enriched. In reared fish, lipid metabolism pathways were the most enriched in all areas. For proteome the enriched pathways were various amino acid metabolism. Only GoF showed significant enrichment between wild and hatchery reared salmon. Between areas wild salmon of BMB vs BS and reared fish of BMB vs BS and GoF showed significant enrichment. Cell cycle and apoptosis and lipid metabolism related transcription factors were the most abundant in all comparisons. There was high correlation in the variances of POPs with both transcriptome and proteome data. The highest number of transcripts were associated to non-dioxin-like PCBs and PBDEs, and proteins to PBDEs and chlordane. In general the difference caused by areas were bigger than those between wild and reared salmon.

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Toxicity evaluations in medaka embryos exposed to chlorophenols using GC/MS metabolomics and explorations of its differences by chlorination position
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Chlorophenols are one of the most common chlorinated aromatic hydrocarbons in the environment, because they are anthropogenically discharged in industrial wastes and are also generated by the degradation of chlorinated pesticides. But there have been few researches on the effects in aquatic organisms. Metabolomics can provide information about the metabolic processes that are occurring in an organism and have proven to be a highly sensitive method even for detecting the effects of toxicants. Previous studies have suggested that metabolite variations can indicate toxic exposure earlier than typical measurable endpoints such as growth inhibitions. In this study, we exposed CPs such as 2,4-dichlorophenol (24-DCP), 2,6-dichlorophenol (26-DCP), 3,5-dichlorophenol (35-DCP), and 2,3,4-trichlorophenol (234-TCP) to the embryos of Japanese medaka (*Oryzias latipes*), and evaluated their effects during the embryonic development using GC/MS metabolomics. Additionally, the differences of toxicities by chlorination position on CPs were examined with metabolomics approach. Medaka embryos were exposed to individual CPs such as 24-DCP: 6.25 mg/L, 26-DCP: 6.25 mg/L, 35-DCP: 2mg/L, 234-TCP: 0.16 mg/L, and each 1/10 of their concentrations, respectively. Embryos were kept in test water spiked with CPs (n=6). Control group, in which embryos were kept with CPs free water, was also established. Embryos were collected at 3, 4, and 6 days after the start of the exposures. Metabolites were extracted from embryos and mesured by GC/MS. The individual areas obtained from all metabolites detected on a GC/MS chromatogram were analyzed by ANOVA among each exposure and control groups. Metabolites with significant differences in ANOVA were selected, and their areas were followed to analyze by principal componet analysis (PCA). CPs exposures had caused several effects in embryos and hatching larvae. Metabolomics approach shows that the toxicities have already appeared at 3 days after the start of the exposure. PCA score plot at 6 days showed that the order of severity of each toxicity and that was 26DCP< 24DCP< 35DCP< 234TCP. As a result, the increase of the number of chlorine could cause more severe toxicity. As a result, the CP toxicities were suggested to be decided by the number of chlorine and the chlorination position.

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Poster spotlight

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Life Cycle Impact Assessment (I)

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Product Biodiversity Footprint – A novel approach to compare the impact of products on biodiversity combining Life Cycle Assessment and Ecology
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Product impacts on ecosystem quality have long been addressed by the top-down approach known as Life Cycle Assessment (LCA). Impacts are most of the time assessed within the "biodiversity loss" damage category indicator. However, LCA methods do not cover the 5 drivers of biodiversity loss as identified by (Millenium Ecosystem Assessment, 2005) (MEA): only land occupation and transformation, pollution, climate change are covered, species overexploitation and invasive species are not. Besides, ecologists work on the ground to measure concrete impacts from given practices on biodiversity in a given area, in a more bottom-up approach, for some parts of the value chain of the product (e.g. production of agricultural bio material). The Product Biodiversity footprint (PBF) approach aims at bridging the gap between LCA and ecology. Its objective is to allow comparison of variants of a product for eco-design, being to our knowledge the first method to address the five drivers of MEA. The methodology combines LCA and ecology and organizes them towards practical indicators and representations for business decision. The general architecture is represented by three modules : i) Module 1 computes life cycle impact assessment; ii) Module 2 refines the quantification of the pressure on 'habitat change', using specific information on practices including ecological data; this second Module, combined with Module 1, allows comparison along the value chain for MEA drivers from habitat change, pollution and climate change ; iii) Module 3 qualitatively assesses aspects that are not included in LCA models, with 'species management' and 'invasive species' indicators, allowing comparison for those two remaining MEA drivers. PBF has been tested in three business case studies. It demonstrates that the integration of ecological data enables to specify LCA results and suggests that current LCA methods tend to overestimate impacts on biodiversity caused by agriculture when "biodiversity-friendly" practices are implemented. However, this method is dependent on the available data/literature connecting specific practices and biodiversity, calling for streamlined data gathering as a potential next step. The method and the main outcomes of the case study conducted with L'Oréal on a simplified shower gel will be presented.

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Incorporating habitat fragmentation into the characterisation of land use impacts on biodiversity

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For the credibility of LCA, it is important to cover the most relevant impact pathways adequately. Since habitat loss is the main driver of global species loss, it is crucial to appropriately characterise land use and land use change impacts on biodiversity. However, virtually none of the currently operational LCIA models considers spatial habitat configuration and fragmentation aspects (but see Larrey-Lassalle et al. 2018), which are recognised as important factors for the survival or extinction of species. Including fragmentation and the spatial composition of the landscape is especially relevant when the landscape (e.g., ecoregion) consists of a distribution of various hospitable habitats, separated by hostile land cover types. We explore methods to quantify habitat loss and fragmentation impacts conforming the LCIA framework such as the fragmented landscape species- area relationship (FL-SAR; Rybicki & Hanski 2013), the countryside species-fragmented area relationship (c-SFAR; Larrey-Lassalle et al. 2018), and methods based on field monitoring data. Furthermore, we propose a new regionally and taxonomically specific impact model with global geographical coverage that quantifies the regional impacts of habitat loss and fragmentation on groups of species that differ in traits relevant for responses to habitat change in multi-habitat landscapes (e.g., habitat preferences, home ranges, and dispersal capacities). Separating the taxonomic groups based on species traits allows for a tailored calculation of the impacts of loss and fragmentation of various land cover types on biodiversity. Subsequently, these regionalised (gridded resolution) and species-category specific impacts are aggregated to broader taxonomic groups (e.g., mammals or birds) and bigger spatial units (e.g., ecoregions). The resulting spatially and taxonomically differentiated characterisation factors will be of high value for regionalised LCIA land use impact modelling. **References:** Larrey-Lassalle P, Loiseau E, Roux P, et al (2018) Developing characterisation factors for land fragmentation impacts on biodiversity in LCA: key learnings from a sugarcane case study. Int J Life Cycle Assess 23(11): 2126-2136. Rybicki J, Hanski I (2013) Species-area relationships and extinctions caused by habitat loss and fragmentation. Ecol Letters 16: 27-38.

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Relevance of considering LCA and ecological expertise methods to evaluate biodiversity impact of green roofs

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Human activities are inducing the sixth Earth's biodiversity mass extinction due to the global acceleration of species disappearance. As a response to biodiversity erosion, more and more urban planners are preconizing the integration of green roofs in European urban projects. Although these initiatives are welcomed, the objective assessment of their actual impacts is rarely carried out. One can distinguish between "*in situ*" biodiversity impacts (impacts on the construction site) and "*ex situ*" biodiversity impacts (impacts due to the extraction of the raw materials, their transformation, etc.). Usually, the assessment of the "*in situ*" impact on biodiversity is carried out based on the ecological expertise method. This method consists of performing a site's state by naturalistic inventories before and after a perturbation. Meanwhile, LCA is recognized by the European Commission as the best tool to assess the environmental performance of a product along its entire life cycle and enables to assess both "*in situ*" and "*ex situ*" impacts. However, LCA "*in situ*" biodiversity indicator is not in accordance with site observations and do not reach a scientific consensus. Improvements need to be made, especially to correct the lack of local specificities' consideration. The aim of this study is to evaluate the biodiversity footprint of two types of green roofs located in Paris: extensive and intensive green roofs. This study owes its originality into the calculation of the overall impacts of green roofs on biodiversity. A correction of the LCA "*in situ*" biodiversity indicator is proposed through the use of ecological observations. This study proves the relevance of considering both LCA and ecological expertise approaches to evaluate the biodiversity. Depending on the approach chosen, conclusions about the overall biodiversity impact (i.e. "*in situ*" + "*ex situ*" biodiversity) are different. According to the LCA approach, the extensive green roof induces less biodiversity loss than the intensive one. When replacing the "*in situ*" result calculated by LCA method with the one calculated with ecological obervations, the difference between the two green roofs is not significant and thus, their footprints have the same impact order of magnitude. In urban planning, attention has to be payed to not promote the "*in situ*" biodiversity at the expense of the "*ex situ*" biodiversity, and vice-versa.

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Inclusion of spatial configuration in LCA by a measure of the pressures on the land use's context -- the case of quarries worldwide

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The need to draw a land use map which includes measures of fragmentation and landscape configuration has been previously identified by the life cycle assessment (LCA) community. The inclusion of fragmentation in LCA has been limited to ecosystems with natural vegetation cover of less than 30% and applied to forest ecoregions. Other forms of landscape configuration caused by anthropogenic activities can occur also in areas with higher habitat coverage, such as perforation (e.g. mines in remote areas). In such situations, the land use area is often negligible compared to other major land uses such as agricultural land, and are consequently overlooked in LCA. So far LCA biodiversity characterisation factors do not explicitly account for the environmental quality of habitats in the immediate surroundings of a land use activity. Here we attempt to close this gap by considering the context surrounding the land use in question. Due to the difficulty in explicitly assessing habitat suitability consistently and at global scale we propose the use of the cumulative human pressure in the surrounding area as a proxy for its habitat quality. Here we use the Human Footprint Index map (HFI) assuming that, generally, for lower human pressures higher will be the habitat quality and lesser will be the habitat fragmentation. The proposed modelling procedure is as follows: 1) for a given land use activity, the spatial data for the location of these activities is obtained using geospatial information systems 2) a buffer of 1km outside the area of the activity is created, 3) and the statistics of the HFI for the buffered areas is calculated. We applied the model on more than 114.000 quarries worldwide. We established a conversion to a perforation potential, from 1 to 0, being 1 the maximum perforation and 0 no perforation, with the fit of a smooth curve for HFI from 0 to 11. The model's intended use is as an add-on to other LCA methods as opposed to a standalone impact category for the impact of land use on biodiversity. The goal is to provide an improvement for the quality axis in the LCA land use framework. The model brings value to LCA by allowing an explicit identification of activities which potentially cause perforation, being particularly relevant for mining activities in remote areas

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A regionalised method to assess irrigation-induced soil salinisation in LCA

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Soil salinisation is one of the main causes of soil degradation, adding to other soil quality threats like erosion, compaction and loss of organic matter. Soil salinisation is the result of accumulation of salts in soils (mainly Cl, Mg, SO4, K, Na and HCO3). This may happen through different mechanisms, being one of the main anthropogenic reasons the use of poor quality irrigation water and improper agricultural practices. Soil salinisation becomes a problem for global agriculture when the accumulation of salt in soil exceeds a level that affects soil properties and crop production on the long term. Despite the hazard that soil salinisation entails for agri-food systems, to date this environmental problem cannot be assessed in life cycle assessment (LCA) studies because i) no global life cycle impact assessment (LCIA) method for irrigation-induced soil salinisation has been developed; ii) commonly used life cycle inventory (LCI) databases, such as ecoinvent, miss information on salt emissions to soils. This work fills these gaps by providing: i) worldwide and regionalised characterisation factors (CFs) to assess soil salinisation impacts in terms of soil salinisation susceptibility (midpoint indicator) and crop diversity loss (endpoint indicator). Native resolution of CFs is 5 minutes and they were aggregated at subregion and country levels to facilitate the impact assessment of agricultural processes which exact location is unknown; ii) a LCI tool with information on salts emitted with irrigation water per crop yield (kg salt emitted/kg crop) per country and 160 crops, covering most harvested mass on global cropland, is also provided. The modelling of the CFs was done using world maps on soil properties and literature of crop production in salt-affected soils. The LCI dataset builds on information available in the ecoinvent database and complemented with information on regional water supply mixes, water quality of different water sources and average agricultural practices. In summary, the LCA model developed allows assessing, for the first time in LCA, soil salinisation impacts of different cultural practices, namely distinguishing between crops, irrigation technologies and use of leaching and drainage systems, in any location of the world.

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Process-specific impact assessment instead of region-specific: the case of crop production and AWARE water scarcity factors
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Regionalisation of impacts for water use in LCA is essential, but including regionalisation in LCA inventories, specially for background processes in LCA, is challenging and time consuming, and often data is not readily available. Agricultural processes that require irrigation are the largest water consumers in the world, and often take place upstream of production processes of manufactured goods, hence increasing the challenge for regionalised data collection. Different agricultural products consume water in distinct regions of a country represented by distinct AWARE factors. When sub-national regional information is not available for the inventory, crop-specific factors at the country scale would be a better proxy than generic, or even agri specific factors. Such factors are calculated using data on crop-specific water consumption as a weighting scheme for the annual watershed-based AWARE factors. The results are crop-specific annual and country-scale AWARE characterization factors for 26 crops or different crop groups. The large variation of factors shows the relevance of using such factors when sub-national inventory data is not available. The average difference across 224 countries between the highest and lowest crop-specific AWARE factor is 12.6, with the highest values being 95.9, 77.0, 72.6, 65.9 and 58.7 for Italy, Australia, South Sudan, Argentina and USA respectively. This means that in these regions, the information on the type of crop you are growing can influence your water scarcity results even moreso than the country your activity is taking place in. The calculated factors are used to calculate the water scarcity footprint of maize, rice, soybean and wheat per producing country, in m^3 world-eq per ton of crop produced in country x. When regionalisation of impact assessment is not possible, using crop-specific characterization factors is a good alternative to capture the spatio-temporal variability of water scarcity impacts, although only spatial variation could be integrated here due to lack of temporally explicit irrigation data. This was applied to crop production but could be extended to other water intensive sectors, such as energy or mining.

Towards a Sustainable Development of River-Sea Systems (RSS) and Coastal Areas (I)

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Achieving water quality goals for coastal and estuarine ecosystems: Case study of ambient monitoring in Sinclair and Dyes Inlets in the Puget Sound, WA, USA
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Achieving water quality goals for coastal and estuarine ecosystems requires an effective monitoring and assessment program to provide metrics that can inform effective management water quality. Here we report on an ambient monitoring program within Sinclair and Dyes Inlets in Puget Sound, WA, USA that was established to characterize environmental conditions, assess potential impacts, and track environmental quality trends within the Inlets. A network of water and biota monitoring locations were selected that were co-located near suspected sources as well as ambient marine and nearshore conditions for periodic sampling. Water column stations and effluents from industrial outfalls were sampled seasonally for trace metals, conventional parameters, and toxicity. Indigenous mussels were sampled biennially for contaminant residues of metals and toxic organic compounds. Results showed water quality in nearshore areas appeared to improve after best management practices for industrial process were implemented. Toxicity from exposure to whole effluent samples was not observed and ambient water samples were not toxic to test organisms, except during the presence of toxic algal blooms. Mussel tissue residues were below effects benchmarks at most locations, however areas with elevated levels of contamination were detected.

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Biomagnification of Tantalum through Diverse Aquatic Food Webs
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Tantalum (Ta) is a Technology-Critical Element (TCE) that is growing in global demand because of its use in electronic and medical devices, capacitors, aircraft, and hybrid cars. Despite its economic relevance, little is known about its environmental concentrations and the trophodynamics of Ta in aquatic food webs have not been studied. Invertebrates and fishes from coastal marine food webs representing different climatic zones in northwestern Chile, western Chilean Patagonia, and the Antarctic Peninsula were sampled and analyzed for Ta. The trophic level (TL) of species was assessed with nitrogen stable isotopes (δ15N), and carbon stable isotopes (δ13C) were used to trace energy flow in the food webs. Levels of Ta varied among taxa and sites, with the highest values found in fishes (0.53 – 44.48 ng g-1dry weight) and the lowest values found in invertebrates (0.11 – 43 7.80 n ng g-1dry weight). The values of δ13C ranged from -11.79 to -25.66 ‰. Ta biomagnified in all four aquatic food webs, with slopes of log Ta versus TL ranging from 0.16 to 0.60. This has important implications as little is known about its potential toxicity and there may be increased demand for TCEs such as Ta in the future. Acknowledgements: This study was financially supported by the projects INACH T31-11 and FONDECYT regular 1161504 (G. Chiang), INACH RG09-14 (J. Celis), VRID 216.153.0251.0 of Universidad de Concepcion, Natural Sciences and Engineering Research Council of Canada and Canada Research Chair funds to K. A. Kidd and a Grant-in-Aid for the Strategic Research Base Project for Private Universities (award S1411016) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (S. Kashiwada).

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Mercury concentrations have decreased in a contaminated area - but where has the mercury gone?
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The western part of Limfjorden, Denmark, Nissum Broad along Harboøre Tange was heavily contaminated with mercury during the 1950ies and 1960ies - mainly due to discharges from the pesticide-producing factory, Cheminova. It is estimated that approximately 30 tonnes of mercury were discharged into the Nissum Broad. Investigations during the 1980ies documented highly elevated concentrations of Hg in sediment and biota along Harboøre Tange; concentrations in sediment with up to 12 μg Hg/g dw exceeded the natural background concentrations by more than 3 orders of magnitude at the most contaminated site; eelgrass (up to 1130 ng Hg g^{-1}) and blue mussels (up to 15 μg Hg g^{-1} dw) exceeded natural background concentrations by up to two orders of magnitude. Recent investigations have shown that mercury concentrations in the sediments along Harboøre Tange have decreased considerably since the 1980ies but concentrations in the benthic fauna are still elevated although they have also decreased since the 1980ies. The purpose of the present investigation was to throw further light on the fate of the mercury in the area. Sediment samples were collected frem the deeper part of Nissum Broad on March 12, 2018, and from the coastal areas of the Broad on April 5-6. The sampling sites were selected to match the sampling sites of as closely as possible. The sediment samples were frozen and freeze dried and the mercury content was determined by means of a Milestone Direct Mercury Analyser (DMA-80). Periwinkles *Littorina littorea* were collected from coastal areas along Harboøre Tange and the northern and southern coast of Nissum Broad. Periwinkles from reference areas without any known history of mercury contamination were analysed for comparison. Mercury concentrations in the deeper parts of Nissum Broad were generally lower than or at the same level in 2018 as they were in the 1980ies – thus showing no indication of movement of mercury from the previosly contaminated sediments along Harboøre Tange to the other parts of Nissum Broad. Mercury concentrations in the periwinkles along Harboøre Tange show almost no overlap with the mercury concentrations in the periwinkles from the reference areas and they appear to be useful sentinels to detect mercury contamination. The reduction in mercury contamination since the 1980ies in the sediments along Harboøre Tange has not resulted in elevated concentrations in other parts of Nissum Broad.

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Bioaccumulation of rare earth elements in two sentinel species of the Loire

estuary: the European eel (Anguilla anguilla) and the European flounder (Platichthys flesus)

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Rare Earth Elements (REEs) are a group of 17 metallic elements with close chemical and physical properties: scandium, yttrium and 14 lanthanides with atomic number ranging from 57 (La) to 71 (Lu). REEs are prevalently used in the industry sector indeed they are employed in numerous processes as in the production of supermagnet and luminophore, petroleum catalysis, pigmentation of plastics, metallurgical alloys, nuclear, MRI … The consumption of REEs was evaluated at 120 kt in 2014 and is increasing by 6% each year [1]. Hence these industrial uses are causing REE releases in the environment and particularly in aquatic ecosystems. REEs are becoming emergent contaminants but they are not yet taken into account in the current regulations. REEs are able to bioaccumulate in aquatic organisms and enter in the food chain. Indeed, REE bioaccumulation in aquatic organisms has already been observed in plankton, algae, bivalve, fish and turtles. REE bioaccumulation is worrying because these elements cause toxic effects. In fact, rare earth elements are neurotoxic, embryotoxic, cytotoxic and carcinogenic, moreover they can cause oxidative stress on cell. The Loire River (France) is one of the largest and the last wild river of west Europe. Its estuary from Saint-Nazaire up to Ancenis is characterized by an important anthropogenic pressure with potential REE emit activities as oil refinery, Aeronautic and naval industries, wind turbine industries, hospitals with MRI, coal-fired power plant … The aim of this study were to define REE bioaccumulation in muscles by eels and flounders, two key species of Loire estuary. They were selected because they have an important socio-economic weight, they are consumed by humans and they are benthic, thus living in contact with the sediment (preferential storage area for REEs). REE levels in eels were determined by ICP-MS at two stages of life (silver and yellow). The silver and yellow stages were distinguished by morphological and physiological changes. The adopted sampling strategy allowed to highlight potential spatio-temporal effects (breeding period and emission sources), intraspecific variations (age, sex, size and weight) and interspecific variations. The first results show that female silver eels accumulate significantly more than males for some TRs. They also show that TR concentrations in flounder muscles are significantly higher than in eel (yellow and silver) muscles.

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Seasonal variability of metal and metalloid concentrations in blue mussels from the North and Baltic Seas

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Since the 1980s, blue mussels are sampled annually for the German Environmental Specimen Bank (ESB; www.umweltprobenbank.de/en). The samples are collected in two-monthly intervals at the North Sea sampling sites Eckwarderhörne and Königshafen and in six-month intervals at the Baltic Sea site Darßer Ort. The frozen mussel samples are cryo-milled to annual pooled samples for each sampling site. We investigated seasonal trends of metals/metalloids in the six-/ bimonthly sub-samples of the years 2013, 2015 and 2017. The objectives of this approach of this project should answer the following questions: 1) Are there any significant seasonal trends of the analytes investigated? 2) How does the seasonal variability compare to the concentration ranges covered by the temporal trends over the last decade(s)? 3) Is it possible to select one sampling period per year which would be representative for the annual mean value gathered from yearly pooled samples for future time trend analysis? In blue mussels at the North Sea site Königshafen we found consistent seasonal trends for cobalt, nickel, cadmium, copper and lead, as well as for selenium and arsenic for the years 2013 and 2015. However, in this trend was confirmed only for selenium in samples from 2017. The picture for mussels collected at the North Sea (Eckwarderhörne) was different: samples of 2013, 2015 and 2017 showed a consistent seasonality of exposure to metals/metalloids. In contrast, arsenic did not follow this trend. One remarkable result of our study is that the variability of element concentrations in the seasonal samples of one single year can be in the same order of magnitude as the concentration ranges over many years, i.e. the temporal trend, which is based on annual pool samples. A possible reason for the seasonal pattern can be the annual life cycle of the mussels. However, the concentrations of metals/metalloids in the mussel tissue are not only influenced by intrinsic biological factors but also by the concentrations of the elements in the surrounding water of the mussel. According to the concentration data found during the seasons it is not possible to determine one representative sampling point during the year which gives a comparable information as the annual pooled samples. It is therefore recommended to pool annual samples.

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Sediment water interface release of 16 metal(loid)s under undisturbed and resuspension conditions in Trave Estuary sediments (German Baltic Coast)

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A study is presented that combines different approaches to estimate the risk posed by different Trave Estuary sediments (representative for the study area), which are in hotspots heavily polluted (especially exceeding guideline values for Cd, Pb and Zn) due to historical industrial use. For this purpose i) the dissolved concentrations of the 16 metal(oid)s Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sn, V, Zn and the non-metals C, P and S in the pore water; ii) the binding form of the metal(loid)s in the solid phase and iii) the potential release of the analytes from sediment suspensions under different redox conditions are examined by i) *in-situ* and *ex-situ* pore water sampling using peeper and the recently introduced meso profiling and sampling system (*messy*); ii) sequential extraction following a modified BCR scheme; iii) suspension and manipulation of the sediments in the bio-geochemical microcosm and iv) simultaneous quantification of all analytes by ICP-QQQ-MS. Results show that i) metal(loid)s in contaminated and uncontaminated sediments of the study area are similarly partitioned between the different fractions of the BCR scheme; ii) dissolved concentrations in the pore water are very similar in the different sediments and vary significantly only in the first cm below the SWI; iii) comparable dissolved concentrations and concentration gradients can be obtained by dialyses based i*n-situ* sampling and suction based sampling from undisturbed sediment cores in the lab; iv) many metal(loid)s are released in case of resuspension, with increasing amounts of elements preferentially bound in the non-residual fractions being released at high redox potentials; v) the expected release of all metal(loid)s is low compared to the overall load of the river Trave; and vi) most pristine, fine-grained sediments of the study area pose the highest risk of metal(loid) release in case of sediment re-suspension.

Substitution of Chemicals of Concern: Perspectives and Connections between Analysis of Alternatives, Sustainable Chemistry and Safe-by-design (I)

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Latest developments in the science and practice of alternatives assessment and further research needs

M. Jacobs, University of Massachusetts, Lowell / Lowell Center for Sustainable Production; J.A. Tickner, University of Massachusetts, Lowell / Community Health and Sustainability; R. Simon, University of Massachusetts, Lowell; S. Edwards, University of Massachusetts Lowell / Lowell Center for Sustainable Production

Alternatives assessment (also known as analysis of alternatives) emerged nearly two decades ago to help guide the evaluation and adoption of safer and feasible substitutes for hazardous chemicals of concern. Today, alternatives assessment is being used in a range of substitution and safer chemistry development contexts, including industry chemicals management operations, regulatory programs, procurement activities, voluntary government substitution support programs, among others. Although the general alternatives assessment frameworks used among these different stakeholders address similar process components of an alternatives assessment, there is a recognition within the field that specific methods and tools used in any given assessment need to be flexible, iterative and adaptable as the specific decision contexts widely vary. Academia, consulting firms, non-governmental organizations (NGOs) and government authorities have contributed tremendously over the last five years to the development of new methods and tools to assist with the alternatives assessment process, recognizing this “fit for purpose” need. New developments in the use of methods to assess the intrinsic exposure potential of a given alternative and the consideration of broader life cycle considerations will be described. New resources and tools for addressing data gaps in the hazard assessment component of the assessment will be shared as well as insights from recent decision analysis/decision making research. Key needs to advance the methods and practice of alternatives assessment as elevated during the recent 2018 *International Symposium on Alternatives Assessment* and as outlined in a recent research and practice agenda for the field will be reviewed.

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Case studies on Analysis of Alternatives under the REACH Authorisation process

E. Van Asbroeck, Apeiron-team NV

In this session we will discuss several examples of Analysis of Alternatives for substances of concern for a specific use. Starting from the key functional requirements of the substance and the required performance it delivers in the end-product, leads to a range of potential alternatives to be objectively assessed against clear decision criteria, to arrive at the final substitution choice. Potential alternatives include: (i) drop-in alternative, (ii) alternative technology, (iii) alternative end-product, but also (iv) relocation and (v) stopping the business. We apply a two-step approach. In the first step, alternatives are looked for as broadly as possible, including; by evaluating the reasons of failure of alternatives tested in the past, by checking what competitors are doing, by cross sector assessment of technologies, by inspiration gathered at green chemistry conferences, etc. In the second step, the short listed highest potential alternatives are assessed in detail on the four criteria defined by REACH in the Authorisation process: (i) reduction of risk, (ii) technical feasibility, (iii) availability and (iv) economic feasibility. The case studies demonstrate how the methodology of Analysis of Alternatives under the REACH Authorisation process can be used as a tool to enable substitution, leading to added value for society while avoiding regrettable substitution.

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Innovation in fouling control, from chemical substitution to UV-C systems
J. Hunter, M. van der Zalm, M. Hindmarsh, Akzo Nobel N.V
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Substitution of Hazardous Chemicals: Lessons, Opportunities and Challenges from a Civil Society Organization's Perspective
M. Wang, D. Santillo, K. Brigden, Greenpeace Research Laboratories
Substitution is a vital element of approaches to address hazardous chemicals and wastes. Support for development and implementation of, and further innovation in, environmentally sound and safer alternatives (including clean production, informed substitution of chemicals of particular concern, non-chemical alternatives) is one of the risk reduction objectives of the Strategic Approach To International Chemicals Management (SAICM), as well as a strategic pathway to the overarching EU objectives for a non-toxic environment. Success in practice depends on the collaborative efforts of all relevant stakeholders, each with a unique role to play. In this SETAC-ECHA substitution special session, we will provide thoughts and relevant experience from a civil society organization perspective, on the following topics, in order to contribute to further discussions: - Drivers for substitution - Importance of collaboration among stakeholders - The role of civil societies in substitution and the importance of their technical and professional capability. - The importance and role of supply chain evaluation and management - Information availability and commercial confidentiality: challenges and opportunities - Functional substitution - chemical and non-chemical alternatives: awareness, guidance, assessment and supporting instrument

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Questions

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General discussion with panel and audience

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Concluding remarks for part I and a teaser for part II

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Wildlife Ecotoxicology: From Sub-lethal Responses to Adverse Effects at Individual and Population Level

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Immunomodulative effects of trace metals in small mammals models
D. García-Mendoza, Wageningen University & Research / Toxicology; B. Han, H.H. van den Berg, Wageningen University & Research / Division of Toxicology; N. van den Brink, Wageningen University / Dept of Toxicology
The effect assessment of immunotoxic contaminants, e.g. trace metals, in environmentally exposed animals is relevant particularly in wildlife species of small mammals that act as hosts for zoonotic infectious agents and modulation of their immune system could play a role in the transmission of such infections. Using a bottom up strategy we assessed the effect of cadmium, a known widespread contaminant metal with immunotoxic potential, on the murine immune system. Initially, we performed an *in vitro* study using different murine immune cell lines showing that cadmium is cytotoxic and may cause toxic and functional effects in different immune cell types, at sub-cytotoxic concentrations. Innate immune cell models, macrophages (RAW264.7 and NR8383 cell lines) and mast cells (MC/9 and RBL-2H3 cell lines), showed a dose dependent depletion of cellular glutathione; while in cells from the adaptive immune system, T lymphocytes (D10.G4.1 cell line) and B lymphocytes (CW13.20-3B3 cell line), glutathione levels were low and cadmium did not have an effect. In all cell lines tested apoptosis was induced at high cadmium concentrations just below cytotoxic levels. Functional effects also showed differential effects. While in activated macrophages cadmium increased pro-inflammatory effects, by enhancing the production of TNFα and nitrite, opposite effects were observed in mast cells, by decreasing the production of TNFα and histamine. In the T cell (Th2) model, proliferation and production of IL-4 cytokine was increased in activated cells at low cadmium concentrations. At sub-cytotoxic concentrations cadmium did not have significant functional effects on the B cell model in study. In order to increase the level of immune integration, ongoing research will determine the effect of cadmium on the T cell-dependent antibody response using *in vivo* mouse models. The evaluation of such response, described as the best predictor for immunosuppression, is dependent on the activation and communication of different immune cell types, which are differentially susceptible of being affected by the exposure to immunotoxicants. The comparison and correlation of *in vitro* and *in vivo* studies will give insight on the potential effects of the environmental exposure to immunotoxic contaminants, such as cadmium, in the immunity of small mammal species, as well as to provide the most relevant *in vitro* endpoints for immunotoxicological assessment.

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Organic pollutants, rodenticides and metalls in the urban terrestrial ecosystem; the case study Oslo city
D. Herzke, NILU - Norwegian Institute for Air Research / FRAM Centre Tromso; E.S. Heimstad, NILU - Norwegian Institute for Air Research; T. Nygård, Norwegian Institute for Nature Research NINA
Persistent organic pollutants (POPs), perfluorinated alkylated substances (PFAS), siloxanes, chlorinated paraffins, organic phosphoric flame retardants (OPFR), dechloranes, UV filters, rodenticides and heavy metals were measured in the terrestrial urban environment of the city of Oslo, Norway, since 2014. NILU and NINA investigated a terrestrial food chain supplemented by predatory species covering varying feeding behaviours (air, soil, earthworm, eggs from fieldfare, sparrowhawk and tawny owl and in liver of red fox, brown rats and batchers). In general, we found a variety of the investigated pollutants in all matrices, with even being able to point out so far unknown pointsources of pollution within the city. Elevated PFAS concentrations in soil and earthworms were found in locations used for skiing activities as well as industrial activities. In 2016, Bromodiolone and brodifacoum were found in rat- and foxlivers varying between up to 3883 ng/g ww and up to 1072 ng/g ww, respectively. We observed major differences between the concentrations and patterns of accumulation of organic pollutants and metals between the species involved in this study. Levels of organic pollutants, especially PCBs, are much higher in the top predators (eggs of sparrowhawk) than in the other species. On the other hand, metals were much higher in earthworms than in any other species. In terms of quantity, PFAS were present in most of the investigated sample types. Distinct interspecies differences were found. PFOS is the overall dominating compound. PFDoA, PFTriA and PFTeA contribute more higher up in the food-chains (fieldfare, sparrowhawk and tawny owl), but also in rodents. Emerging PFAS were also detected. SCCPs were present in all air, soil, fox, sparrowhawk and tawny owl samples, indicating an ubiquitous distribution in Oslo. MCCPs were only found sporadically, mostly in earthworms and soil. In 2016, besides elevated concentrations in air (average 1533 ng/sampler, we detected the highest sumSiloxane concentrations in eggs of sparrowhawk (11.3 ng/g lw) followed by worms and tawny owl eggs (9.9 and 3.1 ng/g lw). OPFRs were either not detectable, or were found in very low levels at the higher trophic levels in biota. However, in earthworms appreciable levels were found (SumOPFR of 898 ng/g lw), compared to about 10 ng/g lw in sparrowhawk, tawny owl and rat liver, all data from 2016. Based on our findings, the trophic magnification potential of these compounds seems to be low.

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Assessing exposure and toxicity of anticoagulant rodenticides to birds of prey in British Columbia, Canada
J.E. Elliott, Environment and Climate Change Canada / Science and Technology Branch Ecotoxicology and Wildlife Health Division; V. Silverthorn, Saltspring Island; S. Lee, Environment and Climate Change Canada; S. Hindmarch, Fraser Valley Conservancy; V. Bowes, Britsh Columbia Ministry of Agriculture; F. Maisonneuve, Environment and Climate Change Canada
Anticoagulant rodenticides dominate pest rodent control globally. Second generation anticoagulants (SGARs) are persistent, bioaccumulative and toxic chemicals that have become widespread contaminants of terrestrial birds of prey. We present data on concentrations of anticoagulants and autopsy results for 560 raptorial birds found dead or brought into rehab, 1988-2016 in British Columbia. Larger generalist owls, e.g. barred owl (*Strix varia*) and great-horned owl (*Bubo virginianus*), had the greatest incidence of exposure; smaller owls e.g. barn owl (*Tyto alba*) had lower incidence , as did hawks, e.g. red-tailed hawk (*Buteo jamaicensis*) and Cooper's Hawk. Bird eating merlins (*Falco Columbarius*) had lowest incidence of exposure among diurnal raptors. Modeling of autopsy and liver residue data show barn owls to be the most sensitive to SGAR poisoning, while great-horned owl least. Probability of lethal AR poisoning increased above 0.1 ug/g SGAR in liver, and rapidly above 0.5 ug/g. Over the study period, the incidence of exposure to SGARs increased from 1988 onwards, remaining consistently high (above 75%) from 2003- 2009, then declining from 2009-2016, which coincides with introduction of regulatory measures in Canada to reduce usage of the most toxic SGARs. Vehicle collision was the most common cause of death for all raptors, followed by trauma, undetermined cause, emaciation, and AR poisoning, which was more common than window collision.

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Multiple stressors affect bird health: a case study in lead contaminated environments
J. Lidman, Department of biology and environmental science, Umeå university / Department of Ecology and Environmental Science; M. Jonsson, Umea University; . Berglund, Umea University / Ecology and Environmental Science
Birds have long been used as biomonitoring species in contaminated environments and although we have good knowledge on the toxic effects of single compounds from laboratory experiments, the outcome may differ in the field, due to multiple stressors in a natural setting. Also, even one single stressor might have multiple effects on the same target due to direct and indirect pathways. In this study we assessed multiple effects of mining activities on insectivorous birds (*Ficedula hypoleuca*) using hemoglobin (Hb) levels as an indicator for bird health. We used metal accumulation (both essential and non-essential), habitat type, and food availability to explore direct and indirect effects of mining. Contrary to our expectation, Hb levels were not directly related with lead exposure, even though lead is known to affect heme synthesis and lead concentration in birds from the mining area exceeded the suggested threshold for lead toxicity. Instead availability of terrestrial flying insects, connection to water and distance to the mine were more important, indicating that the indirect effects of mining activities and specific food items, in addition to habitat, play an important role in the overall bird health. This study highlight the importance of including multiple co-occur stressors when assessing responses of contaminants in wildlife.

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Parental foraging strategies, mercury load and offspring quality in a coastal breeding gull species
C.S. Santos, Ghent University (UGent) / Terrestrial Ecology Unit (TEREC) - Department of Biology; A. Sotillo, University of Aveiro / Department of Biology; M. Monteiro, Aveiro University / Biology; L. Lens, Ghent University / Terrestrial Ecology Unit Biology Department; S. Loureiro, Universidade de Aveiro / Biology
Mercury (Hg) contamination is known to trigger a wide range of deleterious effects on free-ranging animal populations. Monitoring Hg exposure and effects of Hg in sentinel species such as seabirds is hence important to evaluate Hg risks at the population and ecosystem level. This study aimed to address this topic by studying the implications of parental foraging strategies on the Hg input in eggs and chicks, and how this input connects with offspring development, physiological condition and behaviour in the generalist Lesser Black-backed gull (*Larus fuscus*). To address this, a series of field studies were performed, as well as an experimental study carried out at an aviary facility. Briefly, a population of *L. fuscus* was monitored in Zeebrugge (Belgium, 51º2'N, 03º11'E) between 2012-2015. Eggs and chicks' down feathers and primary feathers were collected to assess their main dietary origin and quality, by determining isotopic signatures of carbon (δ13C) and nitrogen (δ15N), and total Hg burden. Parental input of Hg in offspring quality was then assessed by relating information on parental foraging strategies and Hg load with clutch volume and proxies of chick health (e.g. growth rate, body condition and physiological state). Moreover, in a controlled dietary experiment, carried out at an aviary facility, chicks' body condition, physiological state and behaviour (e.g. begging behaviour) was assessed and related to Hg burden of chicks accumulated via dietary uptake. As expected, eggs and chicks' feathers from parents with a stable isotope signature that suggested a predominantly marine diet had higher levels of Hg. Increased maternal input of Hg by females during egg-formation period was negatively related with maternal investment in clutch volume and positively related with Hg load in newly hatched chicks. The latter, in turn, tended to be positively related to DNA damage and negatively related to body condition of chicks. Moreover, increased input of Hg during post-hatch period was associated with a decrease in early development and physiological condition of the chicks. Taken together, our results suggest that the use of marine sources increased maternal and parental input of Hg which may have negative effects on offspring quality.

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Poster spotlight

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Aquatic and Terrestrial Plant Ecology: Ecotoxicology, Risk Assessment and Modelling (I)

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Assessing herbicide effects on non-target terrestrial plants in the greenhouse and in the field
R.J. Isemer-Kellner, Bayer Ag / Environmental Safety Ecotoxicology; C. Mihan, D. Jans, V. Ducrot, Bayer AG Crop Science Division / Environmental Safety Ecotoxicology
During the regulatory approval process for herbicides, potential side-effects to non-target terrestrial plants (NTTP) are evaluated. Routine testing in form of standardized lower tier greenhouse studies is used to assess potential risks. Higher tier testing approaches like field studies may be needed to assess whether the identified risks may occur under more realistic conditions in the field. So far no agreed NTTP field study method has been established. We developed a method for conducting higher tier field studies with NTTPs (posters SETAC Europe 2016&2017, presentation SETAC US 2018) and compared the results to standard greenhouse testing. Effects of a sulfonyl-urea herbicide on 10 crop species as surrogates for NTTPs were measured in the greenhouse in a standard dose-response test according to OECD Guideline 227 (Terrestrial Plant Test: Vegetative Vigour Test, 2006). Shoot dry weight of sugar beet was shown to be the most sensitive endpoint. Lower tier studies in environmentally controlled greenhouses are designed to minimize variation in order to allow for a statistically reliable detection of effects. On the down side, these artificial growth conditions tend to overestimate effects of herbicides due to lack of realism. The higher tier NTTP field study method we have developed may be suitable to overcome this gap. Throughout the growing season 2017 (May to September), the same herbicide already used in the greenhouse testing was tested on 11 plant species sown as a seed mixture for establishing artificial plant communities on a test acre in Germany. Three herbicide rates similar to those used in the greenhouse testing were applied. Vegetation cover according to the Londo scale, dry biomass of total vegetation, symptoms of phytotoxicity as well as BBCH stage of the sown species were assessed. The assessed endpoints and species showed different responses to the tested herbicide and variability was high for some endpoints. Community level metrics such as principal response curves cover endpoints for single species and may be most suitable for the risk assessment. Effects of the herbicide under field conditions were less pronounced than predicted by the corresponding greenhouse study confirming the suitability of the proposed field study method for refining the risk assessment for NTTPs. It needs to be discussed carefully, how to deal with higher variability as well as which of the endpoints should be integrated into the future risk assessment.

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Viability and reliability of assessing reproductive traits of non-crop species for risk assessment under greenhouse conditions
A. Duffner, Eurofins Agroscience Services Ecotox GmbH / Terrestrial Ecotoxicology Lab; C. Gimeno, Trialcamp S.L.U.; T. Moser, Eurofins Agroscience Services Ecotox GmbH
Agriculture is the dominating land-use of the EU member states by covering nearly half of the surface area. Using herbicides to reduce weed competition in agricultural areas can adversely affect non-target terrestrial plants (NTTP's) growing at field margins which provide broad spectrum of ecosystem services (MEA 2005). According to the recent EFSA Opinion for NTTP's (2014) one important goal is maintaining the biodiversity of plant species in the agricultural area. It is therefore recommended to include also non-crop species in the testing scheme from the list provided in OECD guidelines (OECD 208 and 227) and to assess reproductive endpoints such as e.g. seed production. The general objective of this work was to assess the viability and reliability of generative traits of non-crop species for risk assessment under greenhouse conditions in two different European zones. Two different experimental setups were tested. Setup 1: For crops and non-crop-species biomass and plant height at the vegetative and generative growing stage and seed production was assessed when growing at 5 different plant densities in untreated soil, respectively. Setup 2: Selected crop and non-crop species from setup 1 growing at a selected plant density were treated with a total herbicide at five non-lethal application rates. Biomass at the vegetative and generative growing stage and seed production was assessed. The non-crop species for both setups were chosen from the appendix of the OECD guidelines 208 and 227. In both setups 6 replicates were used for each treatment. Both setups were conducted in two greenhouses in different European zones (Germany and Spain). In both setups the biomass and seed production will be statistically evaluated and the coefficients of variation for biomass (vegetative vs. generative growing stage) and seed production will be compared between crops and non-crop species. For setup 2 the ER_X values will additionally be presented for biomass and seed production, where possible. References: EFSA PPR Panel (2014): Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 2014;12(7): 3800. Millennium Ecosystem Assessment (MEA) (2005): Ecosystems and human well-being: synthesis. Island, Washington, DC

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Novel methodology for evaluating the ecological impacts of environmentally relevant antibiotic mixtures on agricultural landscapes
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Fresh water resources are under stress as a result of increasing global populations and a changing climate. As a result, the reuse of wastewater has become a valuable alternative to meet agricultural irrigation demands. However, wastewater effluent is a known reservoir for pharmaceutical residues which include potentially dozens of antibiotic compounds. The impacts of antibiotic exposure in

the agro-ecosystem is of particular concern due to the bio-activity of these compounds and key role microbes play in soil-plant systems. Here we use an exposure algorithm to determine a mixture of antibiotics and predicted environmental concentrations (PECs) in wastewater effluents based on prescription data from the United Kingdom. A synthetic wastewater was prepared and spiked at 5 treatment levels (Control, PEC/10, PEC, 10xPEC, & 100xPEC) and used to irrigate spring barley over a full growth cycle (approximately 12 weeks). Over that period, soil samples were collected at various time periods (prior to antibiotic exposure, 16 hours after initial exposure, 6 weeks of exposure, and 12 weeks of exposure) to track changes in microbial community structure and changes in antimicrobial gene expression. Plant growth parameters were evaluated throughout the study including plant density, tiller formation, leaf development, and plant height. Plant density was negatively correlated with antibiotic exposure with a dose dependent response and this difference increased throughout the growth season. The Skygas 2D system, developed at the University of York, was deployed throughout the entire experiment to continuously measure net ecosystem exchange (NEE) of greenhouse gasses including CO_2, N_2O, and methane. Results showed, for example, that significant differences in NEE of CO_2 corresponded with increased antibiotic exposure as the plants matured. Analytical techniques including targeted LC-MS/MS used to track the fate of antibiotics in the soil-plant system, high mass accuracy LC-MS/MS to evaluate changes in the extractable plant metabolome, and GC-MSD for stable isotope analysis were employed. Results of this study show that at environmentally relevant concentrations, a mixture of antibiotics can impact the soil-barley system and alter ecosystem function.

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Effects of pesticide mixtures on algal biofilm structure and function
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Agricultural pesticides may reach surface waters and impact non-target aquatic organisms and key ecosystem functions. Algal biofilms are ubiquitous in aquatic environments, form the base of aquatic food webs, and have an important role in nutrient and carbon cycling. We investigated the effects of pesticide mixtures on the function (i.e., photosynthetic efficiency) and structure (i.e., taxa composition) of algal biofilms, as well as their recovery after exposure, in a laboratory experiment. Pesticides found in the highest concentrations in Swedish streams were selected for laboratory tests (i.e., 6 herbicides and 1 fungicide). Three mixtures containing 3 pesticides with different modes of action were used in a dilution series ranging from observed toxic units (TU) in long-term monitoring samples, i.e. 1xTU to 100xTU. We found a decrease in biofilm photosynthetic efficiency at concentrations > 10xTU. However, in the low exposures we also observed a stimulatory effect on biofilm photosynthetic efficiency after 2 days of exposure. The latter was interpreted as an increased chlorophyll a production in response to stress, also known as the 'greening effect'. The recovery of the photosynthetic efficiency was high (i.e., 97% of the treatments), especially for biofilms that had experienced low pesticide exposures. This recovery suggests that the pesticides caused a temporary downregulation of photosystem II, but not a permanent damage. However, as our TUs are based on weekly average concentrations measured in the field, it is important to consider that peaks might occur during shorter time intervals, thus leading to potentially higher exposure *in situ*. Preliminary results on biofilm assemblage composition show that several taxa were present only in the control treatments (i.e., the green algae *Aphanochaete* and *Closterium* and the cyanobacteria *Lyngbya* and *Pseudanabaena*). This suggests that pesticides act as a selective pressure for tolerant taxa and can alter natural algal assemblages in biofilms.

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Assessment of biomarkers in macrophytes exposed to individual and combined pollutants of agricultural run-off and climate change
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Macrophytes play an important role in nutrient cycling and in physical and community structuring of aquatic ecosystems. Their potential susceptibility to environmental stressors could therefore lead to a disruption of biotic and abiotic interactions in shallow aquatic systems. In addition to endpoints such as growth and mortality, we consider it important to determine a so-called stressor-specific "chemical fingerprint" of the plant species. Eurasian watermilfoil, *Myriophyllum spicatum* (an OECD as a test organism), the pondweed *Potamogeton perfoliatus* and the waterweed *Elodea nuttallii* have been used in our study to determine individual and combined effects of agricultural run-off (ARO, a defined mixture of nitrates, copper and pesticides) and climate warming. Plants from different microcosm experiments testing effects of different concentrations and pulses of an ARO cocktail at ambient and elevated (+4°C) temperature were analysed. Plant biomass and biomarkers such as pigments, anthocyanins, total phenols and elemental stoichiometry were analysed. *E. nuttallii* showed reduced biomass yield at both ambient and elevated temperature (+4°C) in the presence of ARO. All plants showed a more or less distinct change in pigment patterns, with a tendency of increased chlorophyll contents. A significant increase in carotenoids was seen in *E. nuttallii* at elevated temperature. For *M. spicatum*, a significant reduction in polyphenols was seen at the highest ARO concentration at elevated temperature. In our study, we expect an effect of temperature and ARO on different submerged macrophytes, and that the individual and combined stressor-effects would be better seen with our chemical and physiological biomarkers than by just analysing growth parameters.

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Aquatic Plant Exposures - The Complexities of Higher Tier Testing
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Toxicity testing with aquatic macrophytes is a valuable tool in assessing the risk of herbicides to aquatic plants, particularly for instances where *Lemna* spp. may not provide a complete representation of risk for a specific compound. These aquatic plant tests, however, come with a unique set of challenges. One of the main challenges is determining appropriate species to use when designing exposure(s) when the goal is to produce a species sensitivity distribution (SSD). There are several aspects to consider, including whether there is a published guideline or test method for the species, if there are defined endpoints and acceptability criteria, and if the plants are readily available from reputable suppliers. Guidance for laboratory testing of *Myriophyllum spicatum* exists in OECD TG 239. Published literature on the use of alternate species for laboratory or outdoor environmental testing is available, but no formal, validated test methods for these species exist. Therefore, modifications to the OECD TG 239 exposure design are often implemented when alternate species are utilized in a similar single-species laboratory format. However, the parameters referenced in the OECD TG 239 guidance may not represent the ideal conditions for adequate plant growth resulting in an overall data set that does not properly assess potential toxicity. When determining environmental risk for herbicides, there is a progression of testing tiers for aquatic plants, and each tier has associated benefits and challenges which help to determine what kind of testing should be designed for the goals and objectives. Caution should be used when applying acceptability criteria set for *M. spicatum* to alternate species in single species exposures, as there are currently no validated methods for the alternate species. In order for alternative species to meet similar criteria, some modifications may be required to the amount or source of nutrients in the sediment, temperature or lighting ranges, or culturing practices. With regards to longer-term multi-species studies, as there is no specific guidance for the conduct of the study or required criteria, each exposure system needs to be carefully designed based on the intended objectives. Therefore, test duration, exposure duration, number of species required, and monitoring and sampling intervals will potentially vary between studies.

Complex Mixtures in the Environment: Monitoring, Fingerprinting and Assessment (I)

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How do ion mobility and collision cross section benefit non-target identification in environmental studies
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The identification of chemicals in complex mixtures, such as environmental samples is a challenge due to the presence of both natural and man-made compounds, at different level of concentrations. Separation is a key word to be able to detect low concentration chemicals in such mixtures. Ion mobility (IM) coupled to liquid chromatography (LC) and mass spectrometry (MS) is able to separate isomers and to provide an extra physical chemical parameter, the cross collision section (CCS). The set of isomers, tris(1-chloropropyl) phosphate, tris(2-chloropropyl) phosphate and tris(3-chloropropyl) phosphate presented different CCS values, 161.8, 162.1 and 162.5 Å^2, respectively (measured in nitrogen gas). The lowest difference was about 0.2% between the tris(1-chloropropyl) phosphate and tris(2-chloropropyl) phosphate. Thus, even with a low difference of CCS value we are able to make the difference between two isomers based only on this value. A CCS prediction model was developed using a stochastic Projection Approximation method (Monte Carlo simulations). The training set contains 19 pharmaceuticals and pesticides. The first results showed error between 0.3% to 4.1% for most of the compounds (e.g. mirtazapine and cycluron, respectively), 3 compounds had an error around 8% (e.g. tramadol) and the last three compounds had a much larger error, between 18% and 25% (azithromycin and bisoprolol, respectively). These results showed the importance of considering electrostatic

effects in the model. Environmental samples (e.g. sludge, influent or effluent waters) will be considered to verify the applicability of CCS values, but also to know to which extend these values can be of help the non-target identification workflows.

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Comparing mass, retention time and MS2 spectra as criteria for the automated screening of small molecules in aqueous environmental samples analysed by LC-QToF-MS/MS
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The adoption of database screening using high-resolution, LC-MS data is promising as a pre-screening and routine monitoring tool but depends on the ability to perform reliable data processing on a large number of samples in a unified workflow. Strategies to minimize errors have been proposed but automated procedures are rare. High-resolution LC–ESI-QToF-MS/MS was performed for the analysis of aqueous samples by direct injection. Data processing was achieved with software tools written in *R*. A database containing MS^2 spectra of 693 compounds was integrated into the workflow. Standard mixes and a series of 361 samples of river water were analyzed and processed with the optimized workflow. Using the database and a mix of 70 standards for testing, it was found that an identification strategy including i) mass ii) retention time and iii) MS^2 spectral matching achieved a 2- to 3-fold improvement in the fraction of false positives compared to using only two criteria, while the number of false negatives remained low. The optimized workflow was applied to the sample series of river water. In total, 149 compounds were identified. The best strategy for a database screening approach with minimal manual data manipulation is focused on the reduction of false positives, allowing for the screening of hundreds of water samples for hundreds of compounds in a single run.

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Risk-based prioritisation of organic micropollutants in WWTP effluents and surface waters on a European scale
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The EU project SOLUTIONS provided a huge set of LC-HRMS screening data recorded in a harmonized way at about 500 sites all over Europe including the large rivers Rhine and Danube, a lot of small rivers in many different European countries, rain-event-based samples from small agricultural streams, seasonal series of water samples and a large number of wastewater treatment plant (WWTP) effluents from all over Europe. In the present study these samples were assessed for about 1000 organic micropollutants and their contribution to the overall risk for the three Biological Quality Elements (BQEs) algae, macroinvertebrates and fish. Frequently occurring risk drivers and mixtures thereof were identified. Differences and similarities between large and small rivers, different geographical regions, rain-event and base-flow samples have been evaluated. The criteria for target compound selection were (1) previous detection in surface waters or (2) prediction by emission-based modelling to occur at substantial concentrations in European surface waters or to substantially contribute to risks on aquatic ecosystems. In WWTP effluents more than 300 chemicals have been detected and assessed using the Toxic Unit (TU) approach. Particularly for invertebrates, åTUs exceeded 1 in many effluents with up to 5-fold exceedance in some effluents dominated by specific insecticides. For algae and fish in most cases åTU is between 0.1 and 1, which still represents significant risks. Although the assessment revealed a certain degree of site-specificity for the different types of samples, consistent mixtures of risk drivers became obvious. Taking WWTP effluents as an example, risks to algae during the application period in spring were dominated by photosynthesis inhibitors, while outside this time antibiotics and other pharmaceuticals become dominating, which are emitted the whole year.

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Occurrence and distribution of organic micropollutants in water, sediments and snail tissues in freshwater systems of western Kenya
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In the last decades, the uncontrolled use of emerging organic micropollutants including pharmaceutically active compounds (PhACs), pesticides and industrial chemicals led to their increased detection in the environment. Increasing consumption and discharge has led to concerns on the (eco)toxicological effects since these compounds have been shown to cause adverse effects on organisms including acute and chronic toxicity and endocrine disruption even at low concentrations. Monitoring of these compounds has led to detections in various matrices, with concentrations ranging from sub-ng L^{-1} to $\mu g\ L^{-1}$. However, most of these studies have been performed in developed countries with limited information from developing countries. Since chemical monitoring data are hardly available for Kenyan surface waters, the main objective was to determine the multi-compartment occurrence of chemical mixtures in freshwater systems. Specifically, we aim to: 1) identify and quantify organic micropollutants present within the water, sediments and snail phases, 2) determine the chemical activity and toxic units of these pollutants and 3) for the first time, report the occurrence of personal care products and metabolites in Kenya. Forty eight sites were selected for the monitoring of pesticides, PhACs, hormones, preservatives and their metabolites. To access the impact of land use, different sites were sampled including 27 agricultural (maize, sugarcane, rice and tea), 8 urban and 13 natural sites (potentially no anthropogenic influence). Out of the 580 target compounds, 188 and 64 compounds were detected in water and snails, respectively. In snails, oxypurinol showed the highest concentrations (up to 3.3 $\mu g\ g^{-1}$ snail) while acetyl-sulfamethoxazole (up to 23 $\mu g\ L^{-1}$) was detected mostly in the water samples. Acetyl-sulfamethoxazole is a metabolite of sulfamethoxazole which is used to treat several bacterial infections. The snails showed the capacity to act as passive samplers of the contaminants therefore giving a holistic view of chemical status in aquatic ecosystem as compared to grab samples which are time dependent. Further steps will involve evaluation of sediment data, determination of chemical activities, toxic units calculation and pattern analysis. The results of this study will shed light on the occurrence levels and toxicities of the pollutants present within the aquatic system in western Kenya.

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Bioassay-based profiling of steroid- and dioxin-like activities in French river waters using grab and integrative sampling
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Effect-based methods (EBM) are relevant tools to monitor chemical quality of aquatic systems as they provide integrative assessment of contamination components and inform on hazard associated with complex mixtures. In the frame of a French national program aiming at demonstrating the operational performance of passive sampling to monitor priority chemicals in surface waters, we applied a panel of *in vitro* bioassays to investigate 1) the occurrence of steroid- and dioxin-/PAH-like activities, 2) their distribution in the water column using grab sampling and integrative passive sampling. In addition, we looked into the relevance of *in vitro* estrogenicity in the water as compared to established trigger values and *in vivo* assessment in zebrafish. Twenty surface water sites of different anthropogenic pressures were studied. For each of them, both grab (dissolved phase and particulate matter) and integrative sampling (POCIS and Silicone Rubber membranes) were performed over a 15 days period and organic extract of each sample was analyzed with the bioassays. *In vitro* toxicity profiles in grab water organic extracts showed contrasted contamination patterns depending on the time of sampling (T0 and T15) and the studied sites. Estrogenic and PAH-like activities were the most occurent activities, while glucocorticoidic and anti-androgenic ones were detected at specific sites. No androgenic activity was detected. PAH-like activity was associated with both the dissolved and particulate matter phases while endocrine activities were only found in the dissolved phase. At some sites, *in vitro* estradiol-equivalents were found above the generic trigger value considering environmental risk (0.4 ng E2Eq/L). For 7 sites out of the 10 tested *in vivo*, activities were coherent with those predicted by *in vitro* bioassays. The present study highlights the relevance of combining a set of effect-based tools and smart sampling to monitor site-specific contamination of surface water chemical quality and the partitioning of measured activities in the water column. Our approach further includes *in vivo* mechanism-based assays, which enhances the toxicological relevance of the assessment. Comparison of our data with established trigger values for some activities will be discussed. Finally, ongoing chemical analyses of more than 100 priority substances, including estrogenic hormones, will allow the determination of their contribution to the biological activities detected.

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Antibiotics screening assay: strain selection for validation and application to environmental samples
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Chemicals of emerging concern (CECs) are mostly water-soluble chemicals that have just been or are yet to be identified as contaminants, and are often not included in routine monitoring programs. Toxicological and physicochemical data of these substances are usually lacking, complicating risk assessments for environmental and human health. The identification and risk characterization of CECs may indicate the need for new or additional regulation. Antibiotics are CECs as they are pharmaceuticals that are not fully removed from waste water and can exert biological effects, even after transformation. Their input in the

environment is continuous - they are used in many fields such as aquaculture, agriculture, veterinary and human medicine - and made to be biologically active at low concentrations. An indirect consequence for public health of antibiotic presence in the environment is the selection for resistant bacteria. Target analyzed chemicals often explain only a fraction of the effect observed in a specific bioassay. Hence there is a need to identify other potent chemicals responsible for such measured effects. We aim to develop a bioassay that detects the presence of antibiotic-like compounds in (water) extracts and can be used as high-throughput assay in effect-directed analysis. Bioassay-guided identification of antibiotic-like compounds may result in the identification of unknown compounds with antibiotic properties, or metabolites and transformation products of antibiotics. Four Gram-negative and three Gram-positive strains were selected based on increased susceptibility to antibiotics or their particular read-out, such as constitutive expression of luminesence or green-fluorescent protein (GFP). Further, the use of resazurine as indicator of metabolic activity was determined. The bacteria were exposed to serial dilutions of antibiotics and the MIC90 was determined as the minimum test concentration causing ≥90% inhibition of growth compared to a control. Two bacterial strains were selected for further development of a bioassay, that can be used for the identification of antibiotic-like compounds with effect-directed analysis. The available read-outs for these strains are bacterial density and resazurine reduction. In the presentation, we will demonstrate the sensitivity of these strains to antibiotics with high use in the Netherlands (>50,000 users a year). In addition, assay results will be presented with spiked and field samples.

Ecotoxicology of Nanoplastics: Mechanistic Approaches to Understand Their Risk for the Environment and Human Health

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The effect of chemical and biological conditioning on the chemical toxicity of microplastics to Daphnia magna under various exposure conditions.

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Microplastics are a widespread pollutant of concern that are found in aquatic environments across the globe, including in arctic sea ice, coral reefs and deep ocean trenches. There has been increasing scientific research on this topic in recent years which has led to societal and media engagement and ultimately policy and industry changes. The research to date has demonstrated the toxic potential of microplastics in the environment, which usually manifests as a dose-dependent response. This research explores the potential effects of polyethylene spheres (PE) when combined with selected substances of environmental concern (DEET, Triclosan and Diclofenac) on the model organism *Daphnia magna* (water flea). This research compares toxicity under different exposure conditions to ascertain the potential combined (mixture) toxicity effects under realistic (competitive) exposure conditions with both chemical and biological molecules. Corona formation due to biomolecule adsorption onto plastic particles changes their surface characteristics which can affect subsequent biological and chemical interactions. We compare the effect of different chemical and biological conditioning scenarios on PE particles and the combined effects on *Daphnia magna*. Chemicals were selected from different groups to explore a range of potential effects included insecticide (DEET), antibacterial (Triclosan) and pharmaceutical (Diclofenac). The study was broken into 3 main questions aiming to assess any variation in effects due to the sequence and combination of exposures; 1) What effect does competitive binding have on the adsorption of chemical (co-pollutant) and proteins onto the plastic surface when exposing the plastics to mixture of chemical and *Daphnia-conditioned* medium. 2) How much co-pollutant will be adsorbed into the eco-corona of a preconditioned microplastic, therefore increasing the chemical load? 3) Does the protein corona offer a pathway to reduce toxicity by displacing any of the chemicals on a previously chemically conditioned microplastic, therefore decreasing the co-pollutant load? Toxicity effects were classified using a range of standard OECD tests and end points. The effect that the artificial chemical and natural biological conditioning of the microplastics has on chemical adsorption and desorption rates were key elements of this study, to ascertain how contaminated microplastics may form part of a more complex pollution issue in the natural environment.

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Biointeractions and toxicity of microparticles from waste plastics in in vitro and in vivo systems

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Microplastics (MPs) represent a worldwide concern toward human and environmental health due to their intentional or unintentional release. Environmental MPs are irregular in size, shape and composition. These characteristics make the study of the biological impact of heterogenous MPs of extremely high relevance to better estimate the real toxicological hazards deriving from human and environmental organisms exposure. In the present we used micrometer-sized plastic particles obtained by the fragmentation of plastic wastes of a miscellaneous origin. From the collection and treatment of these plastic fractions is possible to achieve a second-generation plastic material in a granulated form, mailnly constituted by micrometer-size granules. The smallest fractions of the plastic granules, considered as representative of the environmental MPs, have been used to perform toxicity tests using *in vitro* and *in vivo* systems representative of an inhalation and aquatic exposure scenarios respectively. The plastic granulates were mechanically grinded in lab to obtain MP fractions of < 100 μm and < 50 μm. MPs where morphologically charaterized by light and electron miscroscopy techniques and by ATR-FTIR spectroscopy. To evaluate the inhalation toxicity, cell viability and pro-inflammatory responses were evaluated in human alveolar A549 cells. To evaluate the toxicity toward aquatic organisms, amphibian *Xenopus laevis* embryos exposed to MPs were scored for mortality, malformations and growth retardation. Embryos were also histologically analysed to evaluated MP-tissues interactions. MPs resulted very irregular in size and shape, and mainly constituted by PE and PP. The effects on A549 cells resulted size-dependent, with the smaller MPs inducing decrease in cell viability and increase in pro-inflammatory cytokine release. MPs did not induce significant lethal and malformation effects in developing *Xenopus* embryos, while some embryos showed abnormal gut coiling. MPs accumulated into the gut, where lesions at the intestinal mucosa level were evident, together with MPs in contact and/or internalized into epithelial cells. The present results highlighted the importance to study the effects and the biological mode of action of environmental MPs, which greatly vary in size, shape and polymer composition. The mechanic stress toward epithelial cells may represent a triggering event for the pro-inflammatory response.

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Nanoplastic trophic transfer and dietary effect using plant and snail in terrestrial ecosystem

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Plastic pollution in soil environment has increased and the concerns about their effects on soil ecosystem are getting attentions in these days. Especially, among plastic wastes, small sized plastic particles like microplastics (MP, < 5 mm) and nanoplastics (NP, < 1 μm) are getting a lot of attentions. Researchers are focusing on the fate, distribution, and ecological impacts of these plastics. In this study, we investigated the adverse effects of NPs on the organisms in soil ecosystem and the transfer of NPs in the food chain containing crop plant *Vigna radiata* and snail *Achatina fulica*. During 14 days, *A. fulica* consumed *V. radiata* which had grown in the NP contaminated soil for 10 days. After exposure, we investigated the adverse effects of NPs on *V. radiata* (shoot and root growth) and the distribution of NPs in plants transferred from soil to plant. We also investigated the impacts of NPs on *A. fulica* (growth rate, food consuming time, and histopathology) and NP ingestion and egestion via food exposure. In results, NPs entered the plants and transferred to snail through food chain. NPs also induced the growth of plant (decrease of diameter of root). In the case of snail, the growth (weight) of *A. fulica* exposed to high concentration of NP significantly decreased and food consuming time of exposed snail significantly increased (high concentration only) from 4th day of exposure. This can be the evidence of disturbance effects of NPs on the motion of snail. Additionally, damaged tissues were observed in the organs from NP exposed snail and NPs were detected in the feces of *A. fulica*under fluorescent microscope. This indicates that indirect or secondary pollution can occur through ingestion and egestion activities of organisms in soil ecosystem. We confirmed that both direct and food exposure of NPs can induce adverse effects of soil organisms and NPs can be transferred via food exposure in soil ecosystem. *Acknowledgement - This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and future planning (2016R1A2B3010445).*

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Environmental risks of nanoplastics? Defining a size threshold for assessing intake of plastic particles in tissues, organs and cells.

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Plastic pollution is a problem of critical environmental concern and comprises the majority of anthropogenic debris in the environment, including macro, micro, and nanoplastics. So far, the definition of macro, micro and nano-plastics is based on a pragmatic choice. According to one of the most commonly used definitions, microplastics are in the range of 5 mm to 1 mm, while fragments below 1 mm are classified as nanoplastics. However, this classification does not entail any ecological or functional justification. Microplastics may be ingested by animals and may have physical effects of various types. Moreover, they may absorb bioaccumulative toxic chemicals that could be released into the intestinal tract, the so called "Trojan horse" effect. However, microplastics are chemically inert particulate material and cannot cross cell membranes and bioaccumulate. So far, there is some evidence that nanoplastics can enter living cells and bioaccumulate. The objective of this study is to experimentally determine at which size threshold plastic particles may be able to cross the walls of the digestive tract of living organisms, and thus enter cells and bioaccumulate. A test is performed with one-week old adult *Daphnia magna individuals*, in glass beakers with 100 ml ASTM medium and polystyrene nanoparticles of 20, 50, 100, 200, 500, 1000 nm diameter, labeled with Fluorescein. The test is performed in duplicate. The approximated concentration of particles in the test medium ranges from about 10^6/ml (1000 nm diameter particles) to about 10^{11}/ml (20 nm diameter particles). The animals are exposed for 48 hours (three specimens) and 96 hours (three specimens) in order to allow the ingestion of plastic particles in sufficient amounts and to be translocated to other tissues and organs inside the animal. *D. magna* are fed with *Pseudokirchneriella subcapitata*. The specimens collected after 48 hours and at the end of the experiment are sacrificed and fixated with formalin. The presence of plastic particles in the digestive tract and in tissues is examined with Confocal Laser Scanning Microscope (CLSM) to evaluate the bioaccumulation capability of different plastic particles. The results of this study may contribute to the development of a more relevant definition to assess the risks of nanoplastics for ecosystem's and human health

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What's on the outside matters - surface charge and transformations affect toxicity of polystyrene nanoparticles to C. elegans
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With increasing concern about the occurence of plastic particles in the environment, the need to identify the physicochemical properties influencing their toxicity has been recognised. Whether materials at the nanoscale are released as particulates or occur through the weathering of larger plastics once in the environment, they are subject to (further) transformations in waste streams and natural systems. Initially, such processes likely occur at the particle surface through the attachment of molecules present in these systems, thus altering the interactions at the bio-nano interface. Investigating particles with varying surface coatings may shed light on the influence of such interactions on observed toxic effects. In this study, we tested the toxicity of 50nm polystyrene nanoparticles with different surface charges on life history traits of the nematode *Caenorhabditis elegans*. Exposures were carried out from egg and nematode lifespan, growth and reproduction measured over time. Differences in the toxic effects of the pristine materials were established. Positively charged (amine coated) particles were found to exert the greatest toxicity, followed by the neutral (unfunctionalised) particles, whereas negatively charged (carboxyl coated) particles did not influence nematode fitness at the tested concentrations. Further, the aim of this study was to understand how the absolute and relative toxicity of nanomaterials with different starting characteristics change once they have been aged in soil pore waters, in particular how environmental transformations may lead to a convergence of observed toxicities as compared to those of pristine forms under standard laboratory test conditions. To address this, in addition to the assays with "pristine" particles, exposures will be conducted with nanoparticles incubated in pore waters extracted from a field soil for a week prior to testing. Dynamic energy budget toxicity (DEBtox) modelling will be applied to analyse toxic responses with regard to resource allocation. The DEBtox approach uses time resolved data to derive toxicity parameters, e.g. the no effect concentration, which can be used to compare the effects of particle surface properties within each test condition as well as across the pristine and aged exposures. Together, this data will be used to provide insights into the influence of surface coatings and changes thereof in natural systems on nanoplastic toxicity and associated processes.

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Poster spotlight

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Fate and Effects of Metals: Advances in Metals Risk Assessment and Regulatory Guidance (III)

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MONITOOL: new tools for monitoring the chemical status in transitional and coastal waters under the Water Framework Directive
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1. Introduction MONITOOL[1] is a European project formed by 17 Partners covering the Atlantic region from the Canary Islands to the Scottish Highlands and Islands, and one Partner from the Mediterranean region. The main driver of this project is to respond to European Water Framework Directive (WFD)(2000/60/EC) demands for the assessment of the chemical status (CS) of transitional and coastal waters. The WFD establishes that the CS of water bodies must be determined by the comparison of the concentrations of priority substances with Environmental Quality Standards (EQSs), mainly based on the collection of spot water samples. Diffusive Gradient in Thin Films (DGT), and passive samplers (PS), in general, are already widely used in investigative monitoring and there is an interest in their use in the context of the WFD. The main barrier hindering the regulatory acceptance of PS is the lack of appropriate EQS: EQSs in water are defined in the dissolved fraction, preventing the use of DGT-labile concentrations. Thus, the overall objective is to adapt the already existing EQSs for water to those for DGTs, enabling their use for regulatory monitoring. 2. Materials and methods In 2018, two campaigns were carried out in winter (rainy season) and in summer (dry season); simultaneous deployment of DGTs and high-frequency collection of spot water samples were carried out. The sampling campaigns were performed in 4 selected sites (transitional and coastal sites) in each of the 8 European regions (32 sites in total). At the estuarine sites, water samples were taken every day in high and low tide during the DGT deployment period (5 days). All partners used the same DGT supplier and followed the same sampling and analysis protocol. Physico-chemical parameters in water were measured every sampling day. Priority metals (Cd, Ni, Pb) and other specific metals (Al, Ag, Cu, Cr, Co, Fe, Mn, Zn) were analysed in waters and in the DGT resins. **3. Preliminary results** From winter campaign, 230 seawater samples and 140 DGT samples from the 8 consortium regions were analysed. Physico-chemical data and metal concentration results are being collected in a common data base. Preliminary statistical analysis were applied to first results to study relationships between metal concentrations in DGT and in spot water samples. In a final step, suitable EQS for DGTs will be calculated based on the statistical relations obtained previously. [1]https://www.monitoolproject.eu/

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Evaluation of Effects Based Methods for Regulating Metals in Aquatic Ecosystems
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The European Union is currently evaluating the use of Effects Based Methods (EBMs) as part of a more integrative strategy for regulating a number of substances of concern under the Water Framework Directive. This paper evaluates the use of thirteen EBMs that have potential for being applicable for the regulation of metals. Each EBM was evaluated with respect to three criteria: a) metal specificity and sensitivity; b) sensitivity to other classes of toxicants; and c) the strength of the relationship between effects measured by the EBM and effects observed at higher levels of biological organization, such as the individual or population level. The evaluation concluded that none of the EBMs evaluated met all three criteria. The current EU approach of regulating metals is based on Environmental Quality Standard (EQS) assessments (where necessary and where possible, corrected for bioavailability). This approach is sensitive only to metals and has strong links to effects at the individual and population level. It does not, however, consider the integrated effects of metal mixtures on aquatic systems. Given the lack of suitable EBMs for metals, one recommendation would be the continued development of bioavailability models for predicting the toxicity of metal mixtures. Such bioavailability-based metal-mixture models may be the most effective way to achieve the goal of a more holistic approach for regulating metals in aquatic ecosystems.

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Correcting for metal bioavailability in European freshwaters: where do the data come from to make this happen?
I.A. Wilson, A. Peters, G. Merrington, wca; A.C. Ryan, Windward Environmental LLC; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology
In this presentation we will outline the process and outputs from a project in which we have collated and assessed data collected by member states for freshwaters across Europe from which bioavailability correction can now be

made. These data can be used for future bioavailability assessments and to provide context for individual member states, especially where river basins cross national boundaries. This project also provides an opportunity to identify gaps in data coverage and allows member states to target and prioritise resources to enable robust trace element bioavailability compliance assessment. A step-wise process was followed to systematically obtain, screen, and quality assess the available data. Data obtained were from EU member states, via various sources including centrally hosted data archives, data compiled by the European Environment Agency (EEA), contacts at environment agencies through-out Europe, and direct contact to environment agencies. Initial data searches focused on obtaining as much information as possible; the targeted information was separated into categories. Essential data were pH, DOC and Ca; desirable data were Mg, Na, K, Cl, SO_4^{2-}, total and dissolved Cu, total and dissolved Ni, total and dissolved Zn and total and dissolved Pb, and added value data were conductivity, hardness, Fe, Al and other trace elements. After compilation, an initial screening was performed in terms of having the primary relevant sample/site, information and the specific 'essential data'. Following the screen, a quality assessment was implemented; this assessment was based on the ECHA guidance on the use of measured data and particularly on requirements for the use of measured data. Initial results show that although large amounts of data are generated on water quality throughout Europe; the public availability of these data, the determinands assessed, and the quality of the data is hugely variable. Where high quality data are available, it can be used for performing bioavailability assessments, which indicate areas that may be specifically sensitive to metals, both within Europe and within member states. Furthermore, by collating all available information, and by retaining datasets with data-gaps, areas where key parameters are either not measured, or are of poor quality, and significantly hampering the bioavailability assessments of waters are identified.

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Validation of Bio-met for the site specific bioavailability normalisation of EQS for copper, nickel, and zinc
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Bio-met is a user-friendly tool which has been developed to simplify the process of adjusting European Environmental Quality Standards for metals in freshwaters for local bioavailability conditions. An important aspect of why Bio-met is that it removes the need for users to have expertise in metal speciation, ecotoxicology, and bioavailability in order to obtain bioavailability normalised results. This is achieved by using look-up tables of previously performed calculations with the tool selecting the most appropriate result from the look-up tables according to the water chemistry conditions. The results which Bio-met provides convert the EQS-bioavailable which is expressed as "bioavailable metal" into a site specific local HC_5 value which is expressed as a "dissolved metal" concentration and can be compared against monitoring data. At the same time, Bio-met calculates the corresponding bioavailable fraction of the measured (dissolved) concentration of the metal. There are a number of ecotoxicity studies which have been performed in field collected natural water samples in order to provide validation evidence for the Biotic Ligand Models (BLMs) which have been developed to predict metal bioavailability for individual species in natural waters. These studies are available for the same species as where used for BLM development, and in some cases also for other species to which the BLMs are applied. This work makes clear the connection between the validation tests using field collected natural waters and the local HC_5 which is calculated by the user-friendly tool Bio-met, by calculating the local HC_5 for the waters used in the BLM validation studies. When the calculation is performed using the full BLMs the EC10 value for each individual species is calculated in order to generate the site specific SSD from which the local HC_5 is derived. Bio-met provides users with the end result only, i.e. the local HC_5 (or bioavailable metal concentration), without providing the intermediate calculations which are used to compile the SSD.

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A framework for metals read-across: a case study with cobalt carboxylate compounds
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Regulations such as the European Union's REACH (**R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emical Substances) Regulation and the US's Toxic Substances Control Act (TSCA) require a greater understanding of the use and disposal of chemicals in the environment. All require greater information and the development of more empirical data regarding the fate and toxic properties of chemicals in commerce and in the environment. Satisfying these goals requires either the development of vast amounts of chemical specific data or the evaluation and estimation of the chemical and toxicological properties of these substances. Couple with this need for data, the increasing pressure to comply with societal desires to reduce the amount of animal testing conducted, and it is clear that alternate approaches must be developed. One potential approach is to estimate the properties of one "target" chemical, based upon information developed for a similar "source" chemical, a "read-across" approach. Metals, in particular metal salts with inorganic and organic acids, present a unique case for application of a read-across approach. For the most part, current regulatory approaches for control of aquatic metals concentrations are based on measured metals concentrations and then, with some exceptions, are based on the operationally defined "dissolved" fraction. Toxicity testing is typically conducted using readily soluble metal salts and test results are evaluated using measured dissolved metal concentrations; resulting regulatory standards are for the measured metal. In general, this approach assumes that the test material will dissociate in water into its cationic and anionic components and that the toxicological effects observed can be attributed to the components or any bioavailable (i.e., dissolved) species formed. Results from these tests are used to interpolate to other metal salts based on the measurement of dissolved measured metal concentrations. The validity of this approach has been evaluated for select cobalt compounds. Chronic toxicity studies were conducted with a range of cobalt-containing salts and results compared with tests performed on a cobalt inorganic salt, cobalt dichloride. A framework for the evaluation of metals salts, in general, will be proposed.

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Derivation of a protective threshold value for silver towards freshwater organisms
K. Arijs, ARCHE
Driven by EU REACH and the EU Water Framework Directive (WFD), the dataset of available chronic freshwater data for silver was re-evaluated using strict quality criteria. In addition, a testing program was established to generate new chronic ecotoxicity data with potentially sensitive species in order to strengthen the species sensitivity distribution (SSD) for silver. Tests were performed with the plant *Lemna minor* (7-day growth rate test, OECD TG 221), the cyanobacteria *Anabaena flos-aquae* (72-hour growth rate test, OECD TG 201) and the rotifer *Brachionus calyciflorus* (48-hour population growth rate test, APHA test procedure 8420). The critical review of chronic freshwater toxicity data for silver and the additional testing has strengthened the SSD for silver (covering 17 species in 12 taxonomic groups). The dataset includes data for cyanobacteria, which were considered to be particularly sensitive to silver (cfr. anti-microbial properties of silver ions). The corresponding reasonable worst-case PNEC is 0.042 μg Ag/L (< 0.45 μm fraction), and is 2.4 times lower than the lowest EC_{10} in the SSD (*P. subcapitata*). The PNEC is considered adequately protective for sensitive freshwater organisms. Furthermore, the silver PNEC is conservative in terms of bioavailability: the selected chronic toxicity data typically reflect conditions of high bioavailability, with hardness and DOC well below median values in EU natural waters. Industry is currently considering the development of a bioavailability model for silver. The resulting revised freshwater chronic PNEC will be included in the REACH registration dossiers for silver and silver compounds, and is being fed into the discussions ongoing at European Commission level, where silver is being considered as a potential priority substance under the EU WFD.

Modelling and Monitoring of Pesticides Fate and Exposure in a Regulatory Context (II)

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How the multi-year FOCUS surface water modelling will change the risk assessment
B. Kind, J. Kleinmann, K. Billau, WSC Scientific GmbH
For the registration of plant protection products in the EU, a safe use with regard to surface water and sediment organisms needs to be demonstrated. The FOCUS work group developed a stepwise approach. At step 3 different models for drainage, runoff and spray drift entry into different surface water bodies were combined. Due to limitations of these models, the modelling period was limited to 12 - 16 months. Currently the possibility to run multi-year simulations at step 3 is discussed and an approach was presented by EFSA (2018). In the present study multi-year runs were performed using AutoPEC SW to evaluate the possible consequences of an extension of the modelling period for the final risk assessment in surface water and sediment. In the draft scientific report EFSA proposed to implement a number of changes to the exposure calculation in surface water and sediment independent of the extension of the run-time. Some of these adaptations like omitting the Pesticide Application Timer (PAT) or using 90^{th} percentile drift deposition for each individual application were applied to exposure calculations. The resulting PEC values used for the aquatic risk assessment are compared and discussed in this presentation. Additionally, various temporal percentiles for the exposure assessment were evaluated using exposure profile analysis conducted with the software PEC Robot to compare the resulting PEC values with the actual exposure over the whole modelling period. It turned out that weather conditions do have an impact on the amount of substances reaching surface water and sediment via drainage and runoff. Hence, the selection of the application dates based on a PAT seems to be reasonable and should be used also for the multi-year

assessment. For calculations with unrealistic application dates (without PAT) it was found that the use of the 80th percentile PEC_{SW} from all simulation years results in a more balanced PEC_{SW} than the maximum PEC_{SW} of all simulation years, since the maximum PEC may overestimate the exposure to aquatic organisms. However, an exposure profile analysis has shown that the 80th percentile PEC_{SW} from multi-year simulations is only protective if there is just a short, single exposure peak while it may not be protective enough when a single peak lasts for a longer period. The selection of an appropriate endpoint from PEC simulations will be discussed.

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Retention of veterinary antibiotics and herbicides in biochar amended tropical soils
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Agrochemicals have long been used as an effective method to optimize food production in tropical regions. However, the improper use of agrochemicals have been found to cause detrimental effects to natural ecosystems and human health due to their tendency to contaminate surface, ground and drinking water. Biochar has been reported to reduce the leaching of agrochemicals in soils. Studies observe that the potential to reduce leaching is determined by the biochar, agrochemical, and soil properties. The aim of this study is to identify biochar materials that will retain agrochemicals from leaching in tropical soils, therefore reducing their environmental impact. Since the sorption capacity of biochar materials vary depending on temperature and feedstock, this study tested the sorption properties of rice husk, miscanthus straw and softwood pellet biochars produced at 700? to remediate three veterinary antibiotics (enrofloxacine, tetracycline, oxytetracycline) and two herbicides (atrazine and diuron) from contaminating three contrasting tropical soils. Batch studies were carried out and leachates were analysed using High Performance Liquid Chromatography (HPLC). Results showed that amongst all three biochars, rice husk biochar displayed the highest absorption for all agrochemicals tested. Tetracycline held the highest absorption distribution coefficient (K_d) of 5.29 m^3/kg amongst the veterinary antibiotics, while diuron held the highest K_d of 1.13 m^3/kg amongst the herbicides studied. In addition, soils amended with rice husk biochar (2.5% w/w) showed an absorption increase for all agrochemicals. This increase was more pronounced for atrazine in biochar amended loam and sandy silt loam soils. The impact of biochar use on the regulatory risk assessment of agrochemicals was explored further by manipulating soil characteristics in the PEARL model using standard groundwater scenarios (Sevilla and Thiva) with properties of these biochars such as organic carbon, bulk density and hydraulic properties. Incorporation of these biochars in the PEARL model resulted in reduced Predicted Environmental Concentrations in groundwater (PEC_{GW}). In conclusion, applying biochar could provide a cost-effective risk mitigation strategy for agricultural stakeholders. Further studies must be dedicated to exploring the sorption effects of biochar in a tropical climate at the field scale.

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Sorption and degradation of diflufenican in the urban environment
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Diflufenican is a herbicide approved for use in agricultural and urban areas. The approval is based on data obtained for cultivated soils only, while limited knowledge is available on its fate in urban areas. Urban soils typically contain low amounts of organic matter and microorganisms – a situation known to decrease sorption and degradation of many herbicides. In the present study, sorption, mineralization and degradation of diflufenican were investigated on laboratory scale, using a typical urban gravel and a cultivated soil sampled in Denmark. Sorption and degradation were studied for diflufenican and its two aerobic metabolites AE-B and AE-0, while mineralization studies were solely for diflufenican. Diflufenican sorbed strongly to the soil ($K_F = 96$) and much less to the gravel ($K_F = 9$), while the two metabolites showed much less sorption than diflufenican ($K_F = 3.8$ and $K_F = 0.63$ in soil for AE-0 and AE-B, respectively, and $K_F = 1.1$ and $K_F = 0.53$ in gravel for AE-0 and AE-B, respectively). Cumulated over the entire experiment, no mineralization was detected in the gravel, while only approximately 10% of the initial diflufenican concentration had been mineralized in the soil. Diflufenican, AE-B and AE-0 all showed a slower degradation in soil than in gravel. AE-B was formed from diflufenican degradation in both soil and gravel. In the soil there were no indications of accumulation of AE-B, however, in gravel, AE-B kept accumulating over a period of 150 days. The data obtained in the present study shows a situation with limited sorption of diflufenican and its two aerobic metabolites in gravel. Furthermore, no mineralization and only a slow degradation could be detected in gravel, giving rise to the concern for an elevated risk of leaching and groundwater contamination in urban areas. This is of especial concern for the highly mobile metabolite AE-B, which formation pattern in gravel showed no indications of reaching a plateau during the 147-days study period.

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How to Design, Implement and Interpret Groundwater Monitoring Studies in an EU Regulatory Context
D. Wallace, Syngenta; P. Sweeney, Syngenta / Environment Product Safety
Regulations in Europe offer a tiered system by which potential impact of the use of an active ingredient on groundwater can be assessed through a variety of differing options; modelling, semi-field and field. With the Tier 1 model and parametrisation thereof becoming increasingly conservative, an increasing number of higher tier evaluations of GW are being conducted and submitted to Regulatory Authorities. As part of the SETAC group, a thorough revision of how different types of monitoring studies could be conducted and the different protection goals was reviewed. However, the SETAC-EMAG group therefore provided an overview of different monitoring study designs but did not provide any practical framework for interpretation or use in the Regulatory risk assessment. An EU monitoring study was conducted to define a 90th percentile EU groundwater concentration as outlined in SANCO/13144/2010 version 3 and demonstrate safe use. Data evaluation reveals that 90th percentile concentrations of S-metolachlor, ESA (CGA354743) and OXA (CGA51202) to be below trigger, however has been rejected as a design principle, and as such the study re-evaluated to FOCUS scenarios. Instead, the current Regulatory evaluation considers only absolute max. Building on the momentum of SETAC-EMAG, we propose a pragmatic but yet conservative interpretation framework which will allow integration to the current regulatory system.

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Comparison of AGDISP-predicted off field deposition for ground applications using various nozzles and operational conditions
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Nearly all pesticide spray applications result in some off-target drift due to wind movement, spray solution characteristics, weather, and applicator decisions. In 2013, BASF conducted a field study to measure off-field drift from ground applications of several compound-nozzle combinations. Based on the evaluation of the data collected from this field study, the US EPA determined appropriate spray buffers required to meet protection goals for effects to non-target plants. In order to evaluate the drift potential of a wider range of nozzles and drift reducing adjuvants (DRA), an analysis that combined wind tunnel experiments with drift modelling was initiated to determine which combinations of nozzles and DRAs would result in off-site deposition less than or equal to “benchmark” setups analysed in the field study. As a first step, BASF conducted wind tunnel studies to gather information on the produced droplet spectrum data for various nozzles and tank additives. The second step of this assessment was to use the droplet spectra from the wind tunnel experiments as inputs to the model AGDISP to predict potential off-target drift deposition. The predicted spray drift depositions were used to assess the nozzles and tank mix combinations for drift vulnerability by comparing the model results to model predictions for the field study benchmark. Using relative comparisons of AGDISP-modelled drift deposition results removed any bias that might have arisen from the wind tunnel conditions at the time of the experiment or that are inherit to the model used (AGDISP). The AGDISP model results of over 3,000 treatments indicate that operational conditions such as windspeed, use of DRAs, boom height, and nozzle type have a significant impact on off-target drift deposition that should be considered as measures for mitigating spray drift. The study demonstrated that results from field, wind tunnel, and modeling experiments can be combined to determine which combinations of nozzles, DRAs, and operational conditions could be used to meet protection goals. It was further demonstrated that relative comparisons of ground application AGDISP model results are an efficient method to assess the impact of various parameters that can be used for mitigating off-target spray drift deposition.

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Poster spotlight

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Fish Model Species in Human and Environmental Toxicology (II)

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The visual impairments of zebrafish larvae induced by 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)
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The underlying mechanisms of adverse effects of flame retardant BDE-47 on neurobehavioral toxicity were highly concerned, and our previous study indicated that the changes of zebrafish locomotion were plausibly associated with their affected visual sense. To investigate the relationship between BDE-47 exposure and zebrafish visual impairments, a series of experiments covering molecular, morphological and behavioural levels were designed and performed. First, we utilized RNA sequencing to seek vision-related responsive genes of BDE-47 exposure, and validated the sequencing outcomes using qRT-PCR. Both results declared BDE-47 could disrupt the expressions of light-sensitive opsin genes which specifically exist in rods and cones of retina, and four mid-short wavelength sensitive opsins (opn1sw1, opn1sw2, opn1mw1, and rho) were most significantly inhibited. The expression changes of opsins indicated an impaired spectral sensitivity to blue-green light and dim light. Tissue sections and immunostaining were adopted to provide morphological evidences for molecular clues. Histologically, disorderly arranged photoreceptor layer (composed of rods and cones) in retina were observed after BDE-47 exposure. photoreceptor layer, bipolar cell layer and ganglion cell layer were thicker with a more sparse cell distribution in BDE-47 treatments than in the control. Furthermore, decreased rhodopsin fluorescence and unaltered fluorescence from red-green double cones were observed, which were consistent with the changes of opsin genes. In the section of behavioural testing, improved OKR and stumili-evoked escape (looming) responses were employed. BDE-47 exposure significantly reduced the larval OKR response to the blue light but not the mid-long waveband lights. Additionally, larval left eyes had fewer movements than the right eyes after BDE-47 exposure. Escape response is an excellent candidate test emphasizing the roles of visual factors in behavior of animals living in real ecosystems. In the results, the amounts of responsive fish significantly reduced with BDE-47 exposure, reflecting BDE-47 impaired larval behavioral capacity with sensing ambient visual stimuli. Our results indicated that BDE-47 exposure impaired zebrafish larval vision (including color vision) development, and further altered larval behaviours guided by vision, which provided adequate evidence to prove that vision system was a novel and urgent toxicological target of environmental pollutants.

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Can environmentally relevant neuroactive chemicals specifically be detected with the locomotor response test in zebrafish embryos?

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Chemicals considered as neuroactive (e.g. certain pesticides, pharmaceuticals, industrial chemicals) represent one of the most diverse groups of bioactive substances recently detected in European river catchments. Even though the environmental presence of these substances raises concerns, the determination of nervous system-specific effects has been limited using *in vitro* tests or conventional endpoints including mortality. Thus, complementary approaches such as neurobehavioral tests with alternative *in vivo* models like the zebrafish embryo (ZFE) have been proposed. Here we present our new study where we found that exposure of 4-5 days post fertilization (dpf) ZFEs to a set of 18 environmentally relevant compounds with various modes of action (MOAs) led to concentration and time dependet behavioral effects. These behavioral alterations were not restricted to chemicals primarily known to target the nervous system but rather comprised of compounds with anticipated non-neuroactive MOAs too. Furthermore, chemicals with an anticipated same MOA did not necessarily provoke similar behavioral phenotypes. Therefore, a clear distinction of mechanisms on the basis of locomotor patterns for the analyzed set of compounds was not possible so far. Our results suggest that the acute MOAs of certain environmental contaminants in ZFEs are not yet elucidated and that some of them may have unknown neuroactive potentials whereas other compounds considered as neuroactive in e.g. invertebrates may not act or act differently on the nervous system in the ZFE. Furthermore, there is a need improve the specificity of the light-dark transition test when aiming at using it as a diagnostic tool. In addition to specificity of locomotor responses, we found that their sensitivity compared to mortality within the same 24-hour time window (4-5 dpf) and even developmental 4- or 5-day exposure (0-4 or 5 dpf) was increased (≥10-fold) for the majority (5/8) of investigated neuroactive compounds but for none of the other substances. Our results substantiate that locomotor behavior can represent a sensitive endpoint for certain neurochemicals.

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Turquoise killifish (Nothobranchius furzeri) as a new model in behavioural ecotoxicology

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Pharmaceuticals are essential for human wellbeing. However, due to their increasing and continuous use, sub-lethal concentrations of pharmaceutical compounds have already been detected in the aquatic environment. As these compounds are designed to elicit specific biological effects at low concentrations, standard ecotoxicity tests are unable to correctly assess environmental risks associated with pharmaceutical pollution. Reproducible tests that are based on sensitive behavioural endpoints and that accommodate a high ecological relevance have been promoted to fill this gap. We assessed the impact of a three-week exposure to the antidepressant fluoxetine on behavioural traits in *Nothobranchius furzeri* (Turquoise killifish). Overall, this study shows that fluoxetine can impact life skills, such as feeding behaviour, habitat choice in a novel environment and antipredator response of *N. furzeri* individuals while effects on basic behavioural traits were less clear. *N. furzeri* has recently been introduced as a novel model organism for standard ecotoxicological tests and now its potential for behavioural ecotoxicology is being explored.

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Copper nanoparticles impair the mechano-sensory based behavior of zebrafish embryos (D. rerio)

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Metal oxide nanoparticles such as copper-based engineered nanomaterials (ENMs) are used in a wide range of commercial products and applications. Herein, we investigated the impact of newly synthesized CuO-ENMs by assessing different mechano-sensory based behavioral responses of zebrafish (*Danio rerio*) embryos (ZFE). As a first step, we composed a CuO-ENM dispersion- and exposure medium to mimic the natural environment of zebrafish by including natural salts and natural organic matter. Larvae were exposed for 4 hours starting at 96 hours post fertilization (hpf) to non-toxic concentrations of CuO-ENMs. Upon exposure, neither locomotion (swimming distance) nor the startle response (escape reaction upon vibration stimulus) were affected. To investigate the impact of CuO-ENMs on rheotactic behavior (counter flow swimming), we developed a multi-channel flow chamber which allows simultaneous analysis of different rheotaxis-related parameters: Larvae exposed to CuO (0.5 - 4 mg/L; Calibrate-ENMs) spent less time within the inlet zone of individual channels, their head was orientated against the flow for a shorter duration and their body was bent over 15° for a longer time compared to the controls or low concentrations (0.125, 0.25 mg/L). Overall, rheotaxis (orientation against the stream and avoidance of the stream) was significantly reduced, which is likely due to the release of Cu-Ions. Our data demonstrates that rheotaxis is a particularly sensitive endpoint for behavioral responses to CuO-ENMs and warrants further investigations to understand potential particle-specific effects. This research is performed as part of the EU project “caLIBRAte - Performance testing, calibration and implementation of a next generation system-of-systems Risk Governance Framework for nanomaterials”.

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Systems toxicology approach for the assessment of imidacloprid neurotoxicity in zebrafish

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Transcriptomic approaches offer a lot of molecular detail and are increasingly being used as part of a chemical toxicity assessment. However, assumptions must be made that the abundance of mRNA corresponds to the abundance and activity of corresponding protein. To address the above limitations we have developed a comprehensive systems toxicology approach to evaluate neurotoxicity in zebrafish larvae. We have chosen the insecticide imidacloprid for this assessment, because if its ability to interfere with the function of the nervous system in fish. Our systems toxicology approach relies on a network model coded in the Biological Expression Language (BEL). BEL is a tool for representing published biological experimental findings in a computable form by coding molecular relationships as BEL statements. We have developed a biological network model describing pathways that lead to neurotoxicity in zebrafish embryos. The network model is composed of 623 observations curated from 90 publications. Biological processes of autophagy, cell cycle regulation, oxidative stress, endocytosis and response to hypoxia have emerged as the major drivers of neurotoxicity in larval zebrafish. Key signaling nodes in the model, such as protein activities, are linked to information about downstream gene expression. Transcriptomic analysis of genes downstream of these nodes can be used to infer activity of the upstream protein – a process termed network scoring. This approach avoids the assumption that mRNA abundance corresponds to protein abundance and activity. Scoring of the network highlights the most affected nodes by a given treatment which leads to mechanistic hypothesis generation and gives a quantifiable measure of network perturbation. Behavioral analysis revealed that zebrafish larvae exposed to imidacloprid move slower in response to light and vibrational stimuli. In agreement with these data, proteomic analysis showed that proteins involved in visual perception and muscle contraction are differentially expressed in exposed individuals. We are now collecting transcriptomic data from zebrafish treated with imidacloprid. These data will be used to score the neurotoxicity network to determine how well the computational model can predict changes measured in behavioral and proteomic experiments. Mechanistic insight gained from computational scoring combined with phenotypical and proteomic analyses provide a comprehensive method for linking molecular events to organ toxicity.

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Effect of chronic low doses of ionizing radiation on central nervous system development of zebrafish
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In the context of an increasing use of radionuclides in medicine, industry and military sectors and after Chernobyl and Fukushima nuclear accidents, the interest for the radiological protection of environment is raising. Some studies have shown an impact of ionizing radiation (IR) on zebrafish development. However, there is limited data on the genetic mechanisms leading to an adverse outcome for early development stages. The goal of this project is to better understand early effects of chronic exposure to low doses of IR on zebrafish development. For that, the study of genetic mechanisms, as well as their consequences on cental nervous sytem (CNS) during zebrafish development was carried out. Fertilized eggs were exposed from the 4-cells stage to the larvae stage in control condition and to two dose rates (5mGy/h and 50mGy/h) of gamma rays emitted by a ^{137}Cs source. The residual radioactive background in the Chernobyl exclusion zone ranges from 0.1µGy/h to 1mGy/h. The effect of IR on embryo development was evaluated by a morphologic analysis, physiological markers measurements and behavior analysis. Together with these macroscopic markers, molecular responses were determined. Gene expression was measured by RNA sequencing. In parallel, these data were validated by RT-qPCR and *in-situ* RNA hybridization. An increase in γ-H2AX foci was found in embryos at 50mGy/h at 24 and 48hpf. Transcriptome analysis was achieved at 24, 48, 96hpf and both dose rates. Among the 35117 zebrafish genes, 782 were found in common between the two dose rates at 24hpf. Among these genes, many were involved in the CNS development. And, a high number of misregulated genes were involved in retinoic acid pathway, a major pathway in embryonic development. An impact on Go Term related to embryo development, neurogenesis, muscle and eye development, heart morphogenesis and hematopoiesis was observed. At 24hpf, embryos perform spontaneous movements controlled by the motor neurons and sensory neurons of spinal cord. This embryonic activity is an integrative parameter of the functioning of a neural network. It is complementary to analyses at the molecular scale. An increase in embryonic activity at 5mGy/h was shown. The transcriptome analysis has shown an impact on some developemental pathways. These genes misregulation are associated with phenotypic disorders such as DNA double-strand breaks, motor and sensory neurons of spinal cord alteration as indicated by embryonic activity.

Life Cycle Impact Assessment (II)

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A comprehensive assessment of the nutritional and environmental trade-offs of "as consumed" foods
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The need for sustainable food choices for the well-being of humanity and the planet is more evident than ever before. However, we currently lack comprehensive metrics that evaluate foods by accounting for both their environmental and nutritional effects. In most food life cycle assessments (LCA), the nutritional value of food is introduced in the functional unit which can be problematic. As nutrition directly affect health, an alternative and more comprehensive method to account for the nutritional value of food in LCAs is to quantify the food's nutritional health impact. This can be achieved by using the DALY Nutritional Index (DANI) as a new impact category in LCA. DANI an epidemiology-based nutritional assessment tool that quantifies the health burden associated with foods in disability adjusted life years (DALYs) based on 16 dietary risk factors. We demonstrated this approach by determining DANI scores for 7,000 food items (48 distinct food categories) in the US diet. The median DANI typically ranged from –56 µDALY/serving (avoided) for health beneficial nuts and seeds, up to 78 µDALY/serving for health-damaging frankfurter sandwiches. We identified the foods with the closest to median DANI score in each of the 48 distinct food categories and determined their life cycle environmental impacts for carbon footprint, particulate matter (PM), water use, and land use. Environmental impacts were established by combining food composition information from the Standard Reference (SR) database (reference flows) and life cycle inventories from Ecoinvent v3.2, World Food LCA Database v3.1, and ESU World food database, and using Impact World+ and accounting for food loss and waste. Carbon footprint estimates ranged from 5 g CO2eq/serving of sugars to 4.2 kg CO2eq/serving meat mixed dishes. PM health burden impacts ranged between 0.007 (sugars) and 2.7 (meat mixed dishes) µDALY/serving, water use between 0.2 (cooked cereals) and 263 (egg/breakfast sandwiches) m3/serving, and land use between 0.004 (sugars) and 3.4 (egg/breakfast sandwiches) ha,yr arable/serving. Comparing the DANI scores with these environmental impact estimates for these foods did not support any correlations suggesting that there is not always a win-win situation. The findings support that there are real trade-offs between environmental and nutritional impacts of foods that must be considered when attempting to establish sustainable diet solutions.

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Global site-specific life cycle impact assessment for particulate matter health impacts
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High spatial resolution becomes increasingly important in life cycle impact assessment (LCIA) where health and environmental impacts depend strongly on the location of emissions. The health impact assessment of Particulate Matter (PM) is such a case, as impacts may change by orders of magnitude based on several site-specific key parameters. Current LCIA approaches distinguish classes of population densities, classes of stack heights and countries of emission as parameters, but generally do not use site-specific factors like wind directions, specific emission heights, specific population densities, emission flow rates, or atmospheric temperatures. Therefore, we present a methodology to calculate site-specific PM characterization factors (CFs) based on modeling of atmospheric pollutant dispersion, quantification of regional pollutant intake and the non-linear effects of PM inhalation on human health. We apply these site-specific CFs to a global site-specific dataset of power plant emissions and compare health impacts against results from non-site-specific approaches. The results show that the site-specific approach reduces uncertainties substantially, helps to direct data collection efforts, and allows to target individual sites for mitigation measures. This is of particular importance as PM is globally the primary cause for pollution impacts on human health.

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Development of a H-Phrase scoring method for ecotoxicity characterization
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The work presented here contains the first results of our development of a H-phrase based approach for eco-toxicity characterization. A sample set of substances was derived by gathering relevant substances occurring in the emission inventory from an industrial cluster in Stenungsund [4]. The corresponding environmental H-phrases (H4-phrases) to the substances emitted were found on PubChem Database. Two different models for converting the H-phrases into numerical values were developed. There are seven different codes and corresponding H-phrases, covering acute and chronic toxicity as well as different severity levels (harmful to very toxic). Each substance may have several phrases assigned to it. In both ranking models, chronic phrases were considered more severe than acute ones. For both models the ranking was translated into a logarithmic scale, i.e. the step from one level to the next was set to a factor of 10. The first method was developed by studying all combinations of H4-phrases that the emitted substances had and separating these variations into different levels. A substance with several phrases gets a higher ranking than a substance with only one phrase. Chronic phrases are also ranked higher than acute ones. A substance with several phrases would receive a higher ranking than a substance with only one. The second method differs from the first one in that it only considers the most severe H-phrase in the ranking. When comparing the ranking methods, the second one, which only considers the most severe H-phrase in the ranking is probably the most useful. It has seven set levels and won't fluctuate when the dataset changes. The structure of both methods should be discussed further.The next step in developing this ecotoxicity method that uses H-phrases might be to compare it to and evaluate it alongside strictly effect-based hazard factors, such as EC50 or HC50.

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The Effect of Sediment Chemistry on Life Cycle Impact Assessment for Metals
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The USEtox Life Cycle Impact Assessment (LCIA) modeling approach for freshwater/coastal marine compartments was originally developed to describe the fate and transport of non-polar organic chemicals. For metals, water chemistry (e.g., pH, DOC, competing cations) plays a significant role in determining metal partitioning. Recent research addressed this issue using the Windermere Humic

Aqueous Model (WHAM) to compute water column K_D values based on defined input parameters for freshwater and marine archetypes for use in USEtox LCIA model simulations. However, the effects of sediment chemistry on LCIA has not been considered. The purpose of this study is to evaluate the effects of sediment chemistry on LCIA for metals using the risk assessment screening-level model, TICKET-UWM, to calculate LCIA Comparative Toxicity Potentials (CTPs) directly for freshwater and coastal marine environmental compartments. TICKET-UWM includes simultaneous consideration of dissolved and particulate phase transport between a well-mixed water column and an (oxic or anoxic) sediment layer, along with detailed chemical speciation calculations for metal binding to inorganic ligands, dissolved (DOC) and particulate organic carbon (POC), and Fe/Mn oxide surfaces; precipitation of metal hydroxides, carbonates, and sulfides; and dissolution kinetics for metal concentrates/massives and metal sulfide precipitates. The model also provides a direct evaluation of toxicity using either Free Ion Activity Model (FIAM) or Biotic Ligand Model (BLM) calculations. Critical metal loading rates from TICKET-UWM risk assessment model calculations were translated into LCIA CTPs as: CTP = 0.5 x Average Depth / Critical Load. TICKET-UWM simulations were performed for copper (Cu) and zinc (Zn) using variations in sediment chemistry (pH, sediment POC, redox conditions). The difference in computed CTP for Cu is attributed to the strong sequestration of Cu in sediments by AVS under anoxic conditions. In contrast, redox conditions in sediments had little or no effect on the CTP for Zn. The modeling approach presented above provides a simple method for linking risk assessment models such as TICKET-UWM to LCIA model calculations. The approach allows non-linear responses associated with metal partitioning (in the water column and sediment) to be considered in developing LCIA CTPs for surface waters.

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Regionalized terrestrial ecotoxicity assessment of copper-based fungicides applied in viticulture
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There are some modelling issues related to the application of LCA to agricultural systems. One issue is related to the ways in which the spatial variability of agriculture resulting from soil characteristics, climate and management practices is considered. Another issue is related to the assessment of impact categories whose impact is spatially variable, which is the case of terrestrial ecotoxicity. Emissions of metals in agriculture have been shown to contribute significantly to the ecotoxicity impact, as do copper-based fungicides applied in wine-growing regions to combat downy mildew. The objective of this study is to evaluate the geographical variability of the terrestrial ecotoxicity impact of copper-based fungicides applied in European wine-growing regions. This involves the development of regionalized characterization factors (CFs) for copper terrestrial ecotoxicity, and then the application of these CFs to a regionalized life-cycle inventory that considers different management practices in four different regions, namely Languedoc-Roussillon, Minho, Tuscany, and Galicia. Two methodological approaches were used to derive CFs: (a) empirical regression models, and (b) WHAM 6.0 according to the soil properties of the Harmonized World Soil Database (HWSD). The CFs were then aggregated at different spatial resolutions—global, Europe, country, and wine-growing region—to assess the uncertainty related to spatial variability at different scales and applied in a case study. The global CF computed for copper terrestrial ecotoxicity is around 3.5 orders of magnitude lower than the one from IMPACT 2002+, demonstrating the impact of including metal speciation. For both methods, an increase in the spatial resolution of the CFs translated into a decrease in the spatial variability of the CFs. With the exception of the aggregated CF for Minho at the country level, all the aggregated CFs derived from empirical regression models are greater than the ones derived from the method based on WHAM 6.0 within a range of 0.2 to 1.2 orders of magnitude. CFs derived from empirical regression models showed greater spatial variability with respect to the CFs derived from WHAM 6.0. The ranking of the impact scores of the analyzed scenarios was mainly determined by the amount of copper applied in each wine-growing region. However, finer spatial resolutions led to an impact score with lower uncertainty.

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Poster spotlight

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Towards a Sustainable Development of River-Sea Systems (RSS) and Coastal Areas (II)

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Evaluation of the occurrence and fate of pesticides in the water/sediment interacting compartments of the Ebro River Delta (NE Spain) and risk assessment for aquatic organisms
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Agriculture is a relevant economic resource worldwide essential to sustain life. However, agricultural activities impact river basins in a way that they contribute to release chemical stressors into the environment, such as pesticides and fertilizers, which may pose a risk for aquatic ecosystems. In this context, the aim of this study was to evaluate the fate of medium and highly polar pesticides in an important wetland area of the Mediterranean region, the Ebro River Delta (NE Spain), to assess the risk that they may eventually pose for aquatic organisms. For this, we investigated the presence of 52 pesticides in water and sediment samples collected in summer, one of the most thriving periods of the rice-growing season. Selected analytes in water samples were determined by means of on-line solid phase extraction-liquid chromatography-tandem mass spectrometry (on-line SPE-LC-MS/MS). In the case of sediment samples, pesticides were extracted by means of pressurized liquid extraction (PLE), followed by extract clean-up with solid phase extraction (SPE), and analysis of the purified extract with LC-MS/MS. Measured concentrations of pesticides were used to evaluate the ecotoxicological risk for aquatic organisms, using the hazard quotient (HQ) approach, and to estimate pesticide adsorption coefficients (Kd). A total of 32 pesticides were detected in water from the Ebro River Delta. Bentazone, followed by propanil and MCPA presented the highest average concentrations (in the µg/L range). Overall, pesticide concentrations were higher in drainage channels than in irrigation ditches, mainly due to a huge presence of bentazone. According to the calculated HQ values, 10 pesticides (azinphos ethyl, bentazone, chlorfenvinphos, chlorpyriphos, diflufenican, irgarol, malathion, oxadiazon, propanil, and triallate) may pose a high risk for aquatic organisms (HQ>10). Concentrations measured in sediments reflect also accumulation of some of the target pesticides in the bed of the drainage and irrigation channels which may also represent an environmental risk. *Acknowledgements-* This work has received funding from the Government of Catalonia (2017 SGR 01404), the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the project BECAS (grant number CTM2016-75587-C2-2-R), and the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 727450.

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Pharmaceutical contamination in a Mediterranean coastal Lagoon: an indicator of complex interactions between societies and wetlands
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This field study assesses the occurrence of emerging contaminants (EC) of the Albufera Natural Park. This protected wetland has as an area of 21120 hectares and it is located 10 km to the South of Valencia City (Spain). It consists of a highly eutrophic coastal lagoon surrounded mainly by rice fields that occupy the primitive marshland. The Turia River, to the north, the Jucar River, to the South, and a network of irrigation channels bring fresh water to the Albufera system. The study was focus on about 30 compounds including Personal Care Products and Pharmaceutical Compounds, including acid, basic and neutral. The interest in detecting environmental contaminants in different matrices (mostly water, soil and sediment) is increasing in the last years. This for their relevant impact on the global ecosystem (1). The selected compounds, in this study, constitute an important block of organic contaminants frequently found for many reasons(2). The results evidenced the presence of many of the selected EC both in water and sediment. The most abundant ones are analgesic and anti-inflammatory drugs (salicylic acid, diclofenac), antihypertensive (furosemide), anticoagulant (warfarin), stimulants (caffeine) and preservatives (parabens). The relation-ship between the presence of these compounds and the anthropic pressure in the area was carefully analysed. **References** [1] Blasco (2008), Impact of emergent contaminants in the environment: environmental risk assessment. Handbook of Environmental Chemistry Vol. 5, pp. 169e188. [2] Anekwe J.Ebele (2017), "Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment". Emerging Contaminants , Vol. 3, Issue 1, Pag. 1-16.
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Analysis of polystyrene microplastics and suspected screening of polymers in environmental samples.
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This work explores the application of different techniques based on mass spectrometry for the analysis of MPLs. (1) The performance of three different analytical approaches based on mass spectrometry -direct analysis in real time (DART-MS), matrix-assisted laser desorption/ionization coupled to a mass analyser time-of-flight (MALDI-TOF), and HPLC-HRMS will be presented and discussed for the analysis of MPLs; (2) An analytical method based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) equipped with atmospheric pressure photo-ionisation (APPI) source operating in negative mode has been developed and validated for the analysis of polystyrene in surface and seawater. PS was selected as a representative polymer because it is one of the most frequently used for plastics production;, and separated by 104 m/z. The chromatographic separation was achieved with a gel permeation column (GPC) using toluene 100% as the mobile phase. (3) The use of suspected screening of polymer residues in environmental samples using the capabilities of HRMS has been explored. The different MS techniques will be compared and discussed for the analysis of MPLs. A new method for the quantitative analysis of PS with instrumental limits of detection of 2 pg and a method limit of detection (MLOD) of 100 ng/L will be presented together with the results to their application to the analysis of 15 samples collected in the Ebro River and the marine bays of the Ebro Delta. Finally, the use of retrospective analysis of full-scan chromatograms acquired by HRMS will be discussed and examples of application will be presented. **References** Cole, M.; Lindeque, P.; Halsband, C.; Galloway, S. C. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 2010, 62, 2588−2597. **Acknowledgements** This work has been supported by the Spanish Ministry of Science, Technology and Universities through the project PLAS-MED (CTM2017-89701C3-1-R). Gabriella Schirinzi acknowledges her pre-doctoral Fellowship BES-C-2015-0018.

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Evaluation of the adsorption capacity of microplastics in water/sediment systems polluted with persistent organic contaminants
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Microplastics (MPLs), defined as plastic pieces of< 5mm are a recognized environmental problem because their low degradability and, due to their size, they can hamper the oxygen exchange and light leading eutrophication processes. Besides,MPLs can enter into marine food web and, finally, into human diet. MPLs can accumulate persistent organic contaminants and pathogens being also an environmental distributor of these ones. The main objectives of this work were to characterise the sorption capacity of commonly found persistent organic pollutants (POPs) including 7 indicator polychlorinated biphenyls (PCBs) and 18 perfluoroalkyl substances (PFASs) by three different types of microplastics (size between 1 and 300 µm) and to carry out these evaluation mixtures in water/sediment systems. The selected MPLs were high-density polyethylene (HDPE), polystyrene (PS) and Polyethylene Terephthalate (PET) due to their high production and uses, low biodegradability in the environment and their increase as a litter in different aquatic bodies. To assess the capabilities of these MPLs in the aquatic environment, the experimental setup was emulating realistic environmental conditions of freshwater and seawater ecosystems. The results evidenced that MPLs can behave as carriers of selected POPs in marine and surface waters after short exposure time. It can be concluded that MPLs of HDPE, PS and PET can adsorb and stabilise residues of POPs that are present in waters/sediment systems. For the same materials, aged MPLs or/and smaller particles present higher adsorption capacities. In general, the adsorption capacity of MPLs for more polar contaminants studied here were PS > PET > HDPE. The major influence observed for the behaviour are the hydrophobic interaction and salting out effect. Afterwards, the isotherms established for selected pollutants showed that Freundlich model was the most suitable although not all the accomplish this model. Finally, due to the sorption capacity of MPLs for POPs, these should be considered as an extra transport vector for these contaminants in the environment once are adsorbed onto MPL surface'. However, more in deep studies are needed to establish the fugacity factor for MPLs with adsorbed POPs since this is the main parameter that regulates their transferability among environmental compartments. Acknowledgements-This work was supported by the Spanish Ministry of Science,Innovation and Universities through the project PLAS-MED(CTM-2017-9701C3).

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Occurrence of metal nanoparticles and fullerene aggregates in wastewater treatment plants, rivers and the littoral zone of Barcelona catchment area
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The last decades have witnessed the raise and the popularization of nanotechnology in many scientific disciplines and, nowadays, many types of nanomaterials (NMs) are already being used as additives, catalysts, ultraviolet filters, plastics fillers or bactericides. The commercialization of consumer products containing NMs such as of titanium dioxide nanoparticles (NPs), cerium dioxide NPs, silver NPs, lanthanum oxide NPs or carbon NMs, leads to the potential emission of heterogeneous mixtures of nanostructured materials to the environment. Anthropogenic NPs are known to reach the aquatic ecosystem and mix with the background of NPs that naturally occur in the submicron fraction of suspended particulate matter. Some studies have assessed the environmental levels of *nanowastes* with models and, less frequently, through environmental monitoring exercises [1-3]. Experimental studies commonly face several difficulties, such as the extremely low concentrations of NMs and the need of developing reliable tools for distinguishing between metal-bearing NPs and ionic species. Overall, the presence of NMs in the aquatic environment is still poorly characterized. In the present work, a sampling campaign was carried out in the Barcelona catchment area to assess the presence of selected NMs. 30 freshwater samples were taken in the Besòs River basin and in the Ebro River Delta. Also, 18 wastewater samples and 10 points of the Barcelona littoral were included. The presence of titanium-, cerium-, and silver-bearing NPs was assessed by Single Particle Inductively Coupled Plasma Mass Spectrometry (sp-ICP-MS) and fullerenes were analysed by liquid chromatography coupled to high resolution mass spectrometry with an atmospheric pressure photoionization source (HPLC-APPI-HRMS). Our results show that wastewater treatment plants effectively remove most NPs that are contained in the influents, although a fraction of NPs is finally discharged into the rivers. Levels of NPs in surface water are commonly above the limits of detection, while the vast majority of seawater samples show a non-significant presence of NPs. The observed concentrations of NPs are highly variable along the course of the studied rivers and their natural/anthropogenic origin will be discussed. [1] Gottschalk, Fadri, et al. *Environ. Sci. Technol.* 43.24 (2009): 9216-9222. [2] Sun, Tian Yin, et al. *Environ Pollut.* 185 (2014): 69-76. [3] Bäuerlein, Patrick S., et al. *Sci Total Environ.* 576 (2017): 273-283.

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Ascidians (Chordata, Ascidiacea) as model organisms for assessing the extent of anthropogenic pollutants in marine environments
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The Mediterranean coastline has been undergoing dramatic changes in the past few decades. Although environmental and governmental agencies invest much effort in general monitoring and protection of this environment, there is a gap in our knowledge of the physiological impacts of current environmental stressors on the marine fauna, and of the possible potential of marine organisms as bio-indicators of environmental health. Sessile marine organisms such as ascidians may provide invaluable information regarding a wide scale of pollutants and stress factors over time, in contrast to common chemical assays. As highly efficient filter feeders, ascidians are well known for their ability to accumulate heavy metals, and to concentrate harmful compounds that are present in low or even only trace concentrations in the water column. The wide geographic distribution of invasive ascidians in both polluted and pristine habitats make them ideal candidates for bio-monitoring a wide variety of marine habitats, and investigating the pathological effects of a variety of stressors. The main objectives of the current study are to explore the potential use of the solitary invasive species *Phallusia nigra*, *Styela plicata*, *Microcosmus exesperatus*, and *Herdmania momus* as biological indicators for micro-plastic, phthalates, heavy metals, and pharmaceuticals. By combining a suit of analytical methods including chemical, physiological, histological and proteomic analysis we aim to locate and describe 'hot-spots' of polluted zones along the Mediterranean and Red Sea coasts of Israel, and further understand the physiological effect of pollutants on benthic invertebrates.

Substitution of Chemicals of Concern: Perspectives and Connections between Analysis of Alternatives, Sustainable Chemistry and Safe-by-design (II)

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Green chemistry as tool to advance safer solvent substitution
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Sustainable Solvent Selection is a hot area of research, and it is desperately needed throughout the chemical industry as more and more traditional solvents come under ever increasing legislative strain. Our H2020/BBI project **ReSolve** (2017-2020, grant agreement *745450*) sets out to replace two widely used but hazardous solvents – toluene and NMP (N-methyl-2-pyrrolidone) – with safer

alternatives derived from non-food carbohydrates.[1] New solvents will be designed and modelled with the aim of omitting parts of the molecular structure that are suspected of causing their toxicity, i.e. aromatic groups and amide groups. The best candidates will be synthesised at first on a bench scale, and will subsequently be assessed for their sustainability, scalability, and performance in a wide range of applications. Importantly, solvents will be both modelled for suspected toxicity pre-synthesis, and then tested in a selection of *in vitro* tests. This talk will look in greater detail at two leading candidates currently under further investigation and scale-up. 1) An inherently non-peroxide forming ether solvent, 2,2,5,5-tetramethyloxolane (TMO), has been synthesized from readily available and potentially renewable feedstocks (acetone and acetylene).[2,3] Unlike traditional ethers, its lack of a proton at the α-position to the oxygen of the ether eliminates the potential to form hazardous peroxides whilst also making it suitable as solvent to replace toluene in radically-initiated polymerisations. TMOs boiling point (112 °C), melting point (

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Substitution and sustainable chemistry: what are key aspects and how to keep the assessment of alternatives fit-for-purpose?
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Sustainable chemistry is a holistic approach for sustainable development considering the entire lifecycle of chemicals and their products including design, raw materials, production and processing, application, recycling and end-of-life. Besides health and environment, social conditions, science, research, technical and economic aspects have to be considered and balanced to enable the safe and sustainable use of chemicals within the capacity-limits of our planet. Today's chemicals management has the responsibility to avoid long-term and irreversible damage to human health and environment while at the same time meeting societal demand through economic efficiency. Sustainable chemistry in practice, needs to keep the assessment fit-for-purpose, targeting the elements of importance at a necessary but manageable level of complexity. However, sufficient data of chemicals is needed to provide acceptably information for the actors with the supply chain to decide whether a chemical is able to perform distinct functionalities and contribute to more sustainability at the same time. The German Environment Agency has developed instruments to measure sustainability aspects of chemicals, mixtures, products and services. The instruments addressing important life cycle steps of chemicals beyond (eco-) toxicological risks and hazards to human health and environment, like mobility, resource demand, greenhouse gas emissions, as well as social implications, and economic performance. The Guide on Sustainable Chemicals and the corresponding IT-tool SubSelect are instruments for producers and operators evaluating sustainability through a set of criteria applicable to substances and mixtures. The SMART 5 tool provides criteria to assess characteristics of chemicals applications like human health and environmental properties, workers protection and safe storage, resource consumption, and economic performance. These instruments are already used within industry. We demonstrate in case studies that these tools contribute to interpret the outcome of alternative assessment and lead to successful and sustainable substitution. Thereby, they contribute to a holistic sustainable chemicals management and consequently help producers and operators to green their chemical portfolio, products and services.

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A systematic approach to use safer chemicals in product design
L. Chung , Clariant
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From substitution to safe-by-design: towards a safe chemicals innovation agenda
J. van der Waals, Dutch Ministry of Infrastructure and Water Management
Regulations are usually the main driver of substitution of hazardous chemicals, but have drawbacks, such as the time needed to establish hazards and risks, and the costs for industry to adapt existing processes and products. A problem is also that substitutes are often drop-ins that are not necessarily much safer. This points to the need for safe design, where safety aspects (in terms of avoiding damage to human health and the environment) are taken into account at the design stage of chemicals and products. Initiated by the Dutch government, the Safe Chemicals Innovation Agenda was developed as a research agenda to serve as guidance for R&D policies at the EU and Member State level. The agenda and its implementation will be discussed.

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Questions

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General discussion with panel and audience

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Wrap-up and closing

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Climate Impacts on Polar and Alpine Ecotoxicology and Environmental Chemistry

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Influence of permafrost disturbances on temporal trends of legacy and emerging persistent organic pollutants in landlocked Arctic char from lakes in the Canadian High Arctic
A. Cabrerizo, Institute of Environmental Assessment and Water Research IDAEA-CSIC; D.C. Muir, Environment and Climate Change Canada / Aquatic Contaminants Research Division; A.O. De Silva, Environment and Climate Change Canada; S. Lamoureux, Queens University / Dept. of Geography and Planning; M. Lafrenière, Queens University / Department of Geography and Planning
Research performed over the past 10 years in the two paired watersheds and lakes (West and East) at the Cape Bounty Arctic Watershed Observatory (CBAWO) has revealed ongoing permafrost disturbances, which are of significant magnitude and importance in West watershed and West Lake even though both catchments are broadly similar. A series of subaqueous slumps over the period 2008-2012 combined with riverine input of suspended solids, particulate organic carbon (POC) and dissolved organic carbon (DOC) from the permafrost disturbances, have resulted in very high turbidity in West Lake. This high turbidity combined with elevated POC and DOC, and other water chemistry parameters, has persisted during 2012-2017 with continued permafrost disturbances in the West Lake watershed. The changes in water chemistry may also apply to inputs and bioavailability of persistent organic pollutants (POPs), associated with the elevated DOC and POC in West Lake compared to nearby East Lake, and to earlier pre-warming conditions. Therefore, our goal was to assess whether these perturbations due to Arctic warming, would affect temporal trends of legacy such as polychlorinated biphenyls (PCBs), organochlorine pesticides (e.g DDTs, HCHs, HCB) and emerging POPs such as perfluoroalkyl substances (PFASs) in landlocked Arctic char (*Salvelinus alpinus*) in the lakes. A comparison with temporal trends of the same pollutants in landlocked Arctic char from other lakes in the Canadian High Arctic was also included.

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Perfluoroalkyl substances and Organophosphate esters at coastal Antarctica
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Persistent organic pollutants (POPs) have long live-times in the environment, accumulate in biota, and have the potential to reach remote regions such as the open oceans and Antarctica. This is especially true for ionizable POPs, such as perfluoroalkyl substances (PFASs) and organophosphates esters (OPEs). Recent work has suggested that PFAS can be transported to Antarctica by atmospheric transport of sea-salt aerosol and subsequent scavenging by snow, overcoming the limited transport by oceanic currents. On the other hand, even though OPEs have been described in the Antarctic Peninsula region, very few measurements of OPEs for surface seawater are available, and there are no measurements of OPEs in plankton. The goal of this study was to determine the presence and the dynamics of PFASs and OPEs, in Antarctic coastal waters. Specific objectives were to evaluate the relative importance of the atmosphere-land-water exchanges as the entry of PFASs and OPEs, focusing on the role of snow deposition and melting as input of these contaminants in Antarctic coastal waters, and the sea surface microlayer amplifying the concentrations of PFAS at the surface ocean. The samples for this study were collected during three different sampling campains at Deception island and Livingston island (South Shetland Archipelago, Antarctic Peninsula). PFAS and OPES surface seawater, snow, SML and plankton samples were pre-concentrated in-situ at the antartic bases, and stored at -20ºC until their analysis in the lab. The analysis of OPES was performed with gas-comatography coupled to mass spectrometry in tandem, while PFAS were analyzed by liquid cromatpgraphy coupled to mass spectrometry in tandem. Seawater from the SML samples had notable differences in concentrations (0.51-3.05 ng L^{-1}) when compared with underlying seawater taken 0.5 m depth (0.03-1.14 ng L-1). Total OPEs ($\sum_{20}$OPEs) concentrations at surface seawater are ranging between 0.6 and 62 ng $L^{-1.}$ In plankton samples, concentrations ($\sum_{13}$OPEs) ranged from 24.80 to 1341 ng g^{-1}. The results show the ubiquity of PFAS and OPEs in the coastal Antarctic ecosystem.

Results will be discussed in terms of the different inputs (oceanic, atmospheric), distance to coast, and role of snow melting amplifying the concentrations. Especially relevant is the demonstration of the amplification of PFAS concentrations in the sea surface microlayer, the implication of which will be discussed.

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Pelagic vs coastal - What are the key drivers of pollutants load in Barents Sea polar bears?
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Barents Sea polar bears exhibit two space-use strategies. The so-called "offshore or pelagic bears" undertaking long annual migrations to follow the ice toward the Eastern part of the Barents Sea and the "coastal bears" staying on land, in Svalbard when the sea ice retreats. Previous studies have highlighted differences in pollution levels with offshore bears generally being more polluted than coastal ones. However, the underlying reasons are currently unknown. It has been proposed that pelagic bears are more active, have greater energy requirements, greater prey intake and thus greater contaminants levels. Alternatively, this may be related to differences in feeding habits, with coastal bears feeding more on less contaminated prey of terrestrial origin and/ or occupying a lower trophic position. Finally, one might expect a pollution spatial gradient related to proximity of source, uptake or transport routes of pollutants. We used activity as a proxy for energy requirements, measured as the duration a bear was in movement. The role of carbon source and diet were investigated by using the isotopic niche ($\delta^{13}C$ and $\delta^{15}N$) as a proxy of the trophic niche. The bear's trophic level was assessed from stable nitrogen isotopes on amino acids ($\delta^{15}N$-AA), using the phenylalanine and glutamic acid as source and trophic amino acids, respectively. Pollutants were also analysed in harp seals from East and West Ice as a proxy for an East-West gradient of pollutants in polar bears food web. Preliminary results suggest carbon source as an important predictor of pollution level. Bears feeding within the marine ecosystem had higher pollutant levels compared to polar bears feeding in terrestrial areas. The trophic niche of coastal bears was much wider compared to the pelagic one, suggesting they are able to target a greater variety of food sources including terrestrial items. Offshore bears may thus be more polluted because of higher proportion of marine preys. Consequently, the trophic niche is likely a key driver of pollution load in pelagic and coastal Barents Sea polar bears. Additionally, PFAS levels were higher in bears using more eastern parts of the Barents Sea and increasing PFAS levels towards East was also observed in harp seals. Finally, activity appears as a minor predictor of pollution load which comes to refute the former hypothesis and thus, energy requirement is likely not a key driver of pollution load in pelagic and coastal Barents Sea polar bears.

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Are chemical pollutants influencing the ability of seabirds to respond to changing ice conditions in the Canadian Arctic?
K.J. Fernie, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health; R.J. Letcher, B. Braune, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; J. Head, McGill University / Natural Resource Sciences; K. Elliott, McGill University / Department of Natural Resource Sciences
Research shows that the exposure of wildlife to chemical pollutants can elicit endocrine disruptive and behavioral effects. Such potential effects may occur in Arctic seabirds that must concurrently deal with rapid environmental changes relating to climate change. Because the endocrine system plays a critical role in allowing animals to respond to environmental stress (e.g., changing ice patterns), endocrine disruption could influence the ability of wildlife to respond to climate change. We are examining such interactions with thick-billed murres (*Uria lomvia*) that feed from ice flows and breed in Hudson Bay, Canada. Reductions in ice result in the birds spending more energy to obtain less fish that may result in poorer reproductive success and chick development. We sought to determine whether a suite of contaminants that bioaccumulates in this species and the influence of climate change have an effect on avian wildlife by limiting their ability to respond to changes in ice availability. In 2016 through 2018, foraging behaviors and movements of > 67 thick-billed murres were tracked with GPS-accelerometers, and concentrations of thyroid hormones, corticosterone, total mercury (THg), a suite of flame retardants (FRs), and per- and poly-fluoroalkyl substances (PFAS) were measured in the plasma of individuals. Levels of all measured PFASs and FRs were consistently low and unrelated to hormones or behavior. However, THg concentrations were associated with circulating triiodothyronine (T3) prior to foraging. In contrast to a "medium-ice" year (2016), in a "low-ice" year (2017), the relationship between T3 and THg was negative. The T3 concentrations of the birds prior to foraging were associated with their foraging behavior; in contrast to 2016, higher levels of T3 were associated with lower diving rates. We found no associations with plasma corticosterone. GPS tracks demonstrated that birds foraged to the north of the colony during incubation when ice was present, then moved to forage to the northwest as chick-rearing progressed when ice was no longer present. These results suggest that birds were foraging near regions of floating ice, which may improve foraging success and reduce diving rates. Based on our 2016-17 findings, we tentatively conclude that THg may influence the ability of these seabirds to adjust to variation in ice cover, and will further examine our hypothesis with 2018 data collected under different environmental conditions. \n \n

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Impact of pollution in the Arctic on avian influenza prevalence and miRNA-155 expression in breeding seabirds
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In our rapidly changing, anthropogenically influenced, world, a variety of stressors are affecting the functioningof organisms. One of the most severely affected areas by global change is the Arctic. Major anthropogenic stressors in the Arctic include: 1) climate change, where temperatures are increasing at twice the global rate[1]and 2) pollution, due to long range transport and release from melting ice caps and permafrost; resulting in uncertainty over subsequent effects. One predicted large-scale effect of climate change in the Arctic is the amplification of infectious diseases, due to increased spread[2]. Here, we present the first data that avian influenza virus is now present in the Norwegian Arctic (Svalbard), indicating an extra challenge to the wildlife living there. We further hypothesise that the immunomodulatory properties of many environmental pollutants in the Arctic may facilitate viral prevalence within these historically naÃve populations. Here we present data to support the hypothesis that pollutants can impact disease dynamics in wild birds.We have done sothrough a combination of field and experimental approaches at the molecular (miRNAs) level.ς

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Poster spotlight

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Aquatic and Terrestrial Plant Ecology: Ecotoxicology, Risk Assessment and Modelling (II)

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Experimental studies to provide long-term data sets for testing population models for Lemna sp. and Myriophyllum spicatum
G. Arts, Wageningen Environmental Research (Alterra) / Environmental Risk Assessment; U. Hommen, Fraunhofer IME; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology; S. Heine, Bayer Ag / Effect modelling; A. Solga, Bayer AG Crop Science Division; S. Taylor, ADAMA Deutschland GmbH
TK-TD (toxicokinetic- toxicodynamic) and population models are considered a promising tool in higher tier risk assessments. Their main merit is lying in their use as a tool in the analysis and prediction of the effects of diverse and dynamic exposure profiles of plant protection products on aquatic organisms. TK-TD models for aquatic macrophytes have been developed and are still being improved. However, long-term datasets are needed for the callibration and validation of these models. These long-term datasets need to cover the growth of the macrophytes over several seasons under realistic environmental conditions. In order to generate such data, a long-term experimental study was set-up in outdoor experimental systems from 2017 to 2019. The growth of *Myriophyllum spicatum* is monitored in a large number of pond baskets installed in an experimental ditch, while the growth of *Lemna* sp. populations is studied in a set of microcosms. The monitoring of biomass (fresh and dry weight) and shoot length or frond numbers is carried out weekly, biweekly or monthly, depending on the season. Apart from the standing crop, seasonal dynamics of recovery growth rates of shoots or fronds over a time frame of 1 month (*Myriophyllum* shoots) or 1 week(*Lemna* fronds) are assessed. In addition, relevant environmental parameters are regularly measured. A high variability in macrophyte growth was observed between replicates over time in both the *Lemna* sp. and the *Myriophyllum spicatum* experiments. The data analysis that will be performed over the next half a year, will focus on answering the question which variables, that have been measured and statistical parameters can be related to this variation. The recovery experiments and the permanently monitored microcosms will probably deliver the best information to the modellers. In the coming months, the datasets will be completed and methods and results will be summarized for the *Lemna* sp. and *Myriophyllum spicatum* experiments 2017 – 2018 (--spring 2019).

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Modelling effects of time variable exposure to mixtures of sulfonyl urea herbicides on Lemna
A.J. Jones, FMC Agricultural Solutions / Institute of Environmental Toxicology;

T. Scown, FMC Agricultural Solutions; A. Samel, FMC Corporation / Environmental Sciences; U. Hommen, Fraunhofer IME; J. Klein, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology
The Tier 1 risk assessment of plant protection products for aquatic macrophytes is usually based on the growth rate inhibition (ErC_{50}) of the duckweed *Lemna sp*. under 7-day constant exposure. In the next Tier, additional species can be tested to reduce species sensitivity uncertainty and/or testing can be conducted under modified exposure regimes to accounty for more realistic exposures (often predicted to be shorter than 7 days). TK-TD models can help extrapolate from the tested exposure regime to the diversity of exposure regimes predicted by the FOCUS surface water modlels. Such models can be used to simulate laboratory tests with time variable exposures, but they can also be coupled with a poulation model to estimate effects on a population in the field of the full FOCUS exposure profile over time. In the project presented here, we used an extensive labaoratory *Lemna sp*. data sets with different sulfonyl urea (SU) herbicides to calibrate and validate a TK-TD model of the growth inhibition. In the next step, validated models for single active substances were combined to predict the effects of plant protection products including two active substances. Laboratory studies with end use products were used to validate the model for the product mixtures. The *Lemna* model used was based on Schmitt et al. (2013). TK-TD is modelled by the use of four parameters, permeability of the cuticula for the given substance, paritition coefficient between water and plant tissue, and ErC_{50} and slope for the logistic concentration response function related to the internal unbound concentration of the active substance. The mixture effect was modelled by introducing a single additional parameter which allows to consider additive, synergistic or antagonistic effects. The *Lemna* TK-TD model could be parameterized to simulate the herbicidal effects of four sulfonyl urea active substances. First analysis indicates that effects of binary mixtures can be modelled under the assumption of concentration addition, which is expected for substances with the same mode of action.

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A Multi-model Approach for Increasing Environmental Realism in Risk Assessment for Plants

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In 2018 the European Food Safety Authority (EFSA) published a Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TK/TD) effect models for regulatory risk assessment of pesticides for aquatic organisms. The EFSA Scientific Opinion focuses, in part, on a suite of models for primary producers that have been developed over the last decade for algae, *Lemna* and *Myriophyllum*. These models have been used to predict (sub)lethal effects of pesticides under untested (time-variable) exposure conditions and the potential for non-target plant populations to recover from these adverse impacts. While each of these models have the potential to incorporate environmental realism in the form of time-variable flow, temperature, irradiance, and nutrient availability, most experiments and tests of the models, to date, have been done under fixed growth conditions as observed in laboratory settings. Since the performance of these models has not yet been characterized for environmentally realistic scenarios, we adopted a multi-model approach to explore how results from these new models compare and complement results obtained using AQUATOX (https://www.epa.gov/ceam/aquatox). While the equations governing the growth of primary producers in AQUATOX do not include mechanistic formulations of TK/TD, the ability to readily incorporate time-variable environmental forcing functions and ultimately trophic interactions into a well-established model are distinct advantages. As part of the "bridging" across the different models, AQUATOX was first setup to mimic FOCUS scenarios with static environmental conditions, analagous to the setups used for the TK/TD models, and parameterized using the same species-specific biological traits, toxicity data and chemical/physical parameters. These AQUATOX FOCUS scenarios were then extended to assess effects associated with time-variable environmental forcing. AQUATOX simulations were evaluated to ensure that reasonable growth trajectories could be simulated across multiple years for the various primary producers that were modeled. Predictions and results from our combined modelling efforts highlight the unique strengths (and weaknesses) of the various models that were evaluated. Our approach illustrates how a multi-model approach can be used to gain insights into single-species, population-level impacts that are more detailed and comprehensive than can be achieved by using either AQUATOX or an individual TK/TD model in isolation.

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Application of functional traits in plant population modelling for chemical risk assessments

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The use of population models in herbicide risk assessments for plants is recommended by the scientific community. However, demographic data to parameterize such models are scarce, especially for plant species of conservation concern. Fortunately, existing global repositories of demographic (DT) and functional traits (FT) data are now available. FT are morpho-physio-phenological traits that influence fitness. Several correlations between DT and FT have been identified. In this study, we investigate how such correlations compare across terrestrial plant species of differing conservation concern and use them in the development of a conceptual model for herbicide risk assessments for data-poor species. COMPADRE is an online repository that compiles transition matrices (currently for 664 vascular plant species) that summarize population demography. Likewise, the TRY database compiles FT data (currently for 69000 plant species). We use transition matrices for 213 species to derive different DT. Using the red list from the IUCN we determined the conservation status for these species, of which 50 are threatened. A Welch's ANOVA was performed to explore differences in demography between plant species of different conservation status. Multivariate analyses were performed to explore similarities and differences among species and to explore links between FT and DT. Our preliminary results indicate that there were significant differences in resilience, population growth rate, mean sexual reproduction, specific leaf area, leaf nitrogen and seed mass between threatened plants and plants with no conservation concern. However, plants of conservation concern and plants with no conservation concern did not significantly differ in traits such as longevity, mean life expectancy, survivorship, age at first reproduction, wood density and leaf life. The conceptual model that we developed consists of a generalized plant life cycle with links between DT/vital rates and FT and that links the effects of environmental variability, demographic stochasticity, disturbances (e.g., drougths) and chemical stressors. In conclusion, species of different conservation status differ in some sets of traits but not in others. This suggests that in some cases we may be able to use data from species of no conservation concern to parameterize population models for data-poor species, but that this will need to be carefully assessed to account for differences in other traits.

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The GraS model - Simulating the vegetation dynamics for a raster-based landscape

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Environmental risk assessment is currently facing high biodiversity losses and a massive decline of insects in our agricultural and semi-natural landscapes. To address these problems, the landscapes complexity needs to be regarded as a whole, including the impact of multiple stressors. Vegetation dynamics play a key role forming the basis of food webs and providing habitats for animals. Especially semi-natural vegetation elements such as grassy field margins and hedges are of utmost importance, as they e.g. provide nesting sites and food sources throughout the year and are crucial for the resilience and/or recovery of populations. Process-based modelling techniques are recommended to cope with this high complexity on the landscape scale. We here present a dynamic, spatially explicit vegetation model that describes (semi-)natural vegetation dynamics using an individual-based model (IBM) for trees and a compartment model with difference equations for the herb layer. Both submodels work and interact in a spatially explicit, raster-based landscape. Input data for the model consist of spatially explicit GIS data on specific site conditions, the surrounding vegetation, the abundance of ungulates and land use (mowing) of a real landscape. The GraS-Model has been used for management decisions in the Eifel National Park (Germany) and for the management of windthrow areas in Germany. Model testing using space for time substitution showed a good correspondence of modelled and observed vegetation dynamics. The chosen approach was successful in modelling competition of species as well as the impact of land use and herbivory. Site conditions (temperature, moisture, soil pH, nutrients) have been included in the current version of the model. The GraS-Model can be helpful to disentangle and understand the influences of various stressors and to show indirect effects. The model will be developed to include the toxicological effect of herbicides in agriculatural landscapes. Simulated vegetation patterns will be used as a matrix for insect populations in order to understand their reaction to multiple stressors including indirect effects such as the loss of flowers in grassy field margins due to nitrogen input and/or herbicides. With this application, we want to address the urgent question, which management measures should be selected to restore biodiversity or to prevent further biodiversity losses.

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The plant community model IBC-grass in ecological risk assessments: A review of recent studies and future applications

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To assess the impact of herbicide applications on semi-natural habitats like field margins, there is an increased demand on tools to extrapolate individual-level effects measured in standardized greenhouse experiments to population and even community level. Main drawbacks of field studies are their reproducibility, defining a reference community and the lack of standardization. Ecological models are frequently mentioned as suitable tools to bridge the gap between individual-level experiments and the protection goals on community-level as several different environmental scenarios can be covered. The plant community model IBC-grass represents a suitable approach to transfer the individual-level effects into a model community in order to estimate the effects at the plant community level. Several recent studies have explored the potential of using an ecological model in future risk assessments. We will present the results of three studies: The first study validated the plant community for short-term level effects on artificial communities by comparing model predictions to empirical data. The second study aimed at comparing the community-level impact if different (individual-level) plant attributes are impaired by a theoretical herbicide, including reproductive attributes such as seed number. Finally, the third study compared the impact of a theoretical herbicide on three plant communities, which differ in the PFT composition, resource levels and disturbances such as grazing, trampling and cutting. These three studies give a first impression of the potential of IBC-grass to be used in environmental risk assessments. We will also give an outlook on potential future model developments and areas in ecological risk assessment and management for which such a model can be a useful tool.

Complex Mixtures in the Environment: Monitoring, Fingerprinting and Assessment (II)

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Modelling mixture effects on the estrogen axis using an OECD related biomarker
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Over recent decades large numbers of chemicals have been identified as interfering with normal functioning of the estrogen axis. It has become apparent that in order to correctly estimate the risk posed by these chemicals then, in addition to identifying the chemical as endocrine active, two additional elements are required. First, an understanding of the link between the measured effect and a deleterious physiological outcome. This prevents overestimation of risk by taking into account natural variations in signalling as well as homeostatic mechanisms. The second of these elements is an understanding of the effects of multiple chemical stressors acting simultaneously, as in a real-world scenario, compared to the profile of a single chemical during a laboratory study. With this in mind we applied a transgenic medaka fish eleuthero-embryonic model, harbouring the *choriogenin h* promoter driving expression of *gfp*, to the problem of defining mixture relationships. The structural role of choriogenin proteins in egg formation suggests that this biomarker may, like the highly related vitellogenin biomarker, not only reveal estrogen axis activity, but may also accurately predict impaired reproduction. Using more complex exposure protocols this model is also capable of narrowing down the mechanism of action of single chemicals. To explore the possibility of synergistic effects following exposure to binary mixtures, we tested pairs of chemicals exhibiting different mechanisms of action likely to result in synergism. In addition we tested one pair of chemicals consisting of two chemicals with the same mechanism of action, expected to give an additive effect. The selected chemicals included pesticides, pharmaceuticals and a plasticiser, therefore fitting the criteria of realistic mixture components for mimicking exposure of humans and wildlife. The different binary mixtures exhibited predicted, synergistic and anti-synergistic effects when compared to concentration-addition and independent action models. Finally, 12 organic water pollutants exhibiting different mechanisms of action and belonging to a variety of chemical classes were tested individually and in two mixtures with differing component ratios. The experimental observations correlated well with the predicted effects of the mixtures. The ChgH-GFP model has therefore been validated for use in determining the mechanism of action of individual chemicals and the combined effects of complex mixtures.

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A novel sample preparation procedure for the combined assessment of occurrence and toxicological effects of micro-contaminants of emerging concern in surface waters.
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Since contaminants of emerging concern (CECs) are present in waters as complex mixtures, target analysis often fails to explain their observed adverse effects on aquatic organisms and human health. Thus, a proper hazard assessment of CECs requires to be addressed by the combination of advanced chemical and bioanalytical tools, as in effect-directed analysis (EDA). A novel sample preparation procedure relying on Solid Phase Extraction (SPE) combining different sorbent materials on a sequential-based cartridge, followed by liquid chromatography coupled to high resolution tandem mass spectrometry (LC-(HR)MS/MS) was optimized and validated for the enrichment of 117 widely diverse (log Kow = [-4-5]) CECs (e.g. pesticides, pharmaceuticals and flame retardants) from surface waters (SW) and further combined chemical and biological analysis on subsequent extracts. A battery of in vitro CALUX bioassays for the assessment of endocrine, metabolic and genotoxic interference and oxidative stress were performed on the same SW extracts. Satisfactory recoveries ([70-130]%) and precision (< 30%) were obtained. Internal standard calibration curves used for quantification of CECs, achieved the linearity criteria ($r2 > 0.99$) over three orders of magnitude. Instrumental detection limits and method quantification limits were of [1-96] pg injected and [0.1-58] ng/L, respectively; while corresponding intra-day and inter-day precision did not exceed 11% and 20%. The developed procedure was applied for the combined chemical and toxicological assessment of SW. Levels of compounds varied from500 ng/L. Artificial sweeteners, industrial chemicals and flame retardants were the most abundant and ubiquitous groups. All SW samples analyzed produced a biological response in one or more of the eight different endpoints measured. Qualitative cause-effect relationships between concentrations of CECs and bioactivity measured in SW extracts were described. The co-occurrence in SW of measured and unknown compounds that might be agonists or antagonists of a certain or multiple bioactivity triggered a complex behavior that could not be disentangled. Thus, further assessment requires to be addressed by an effect-based monitoring combining chemical and bioanalytical tools, as in EDA. Given the demonstrated reliability of the validated sample preparation method, the authors propose its integration in EDA procedures for a proper evaluation of SW chemical and biological quality status.

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Development of an active air sampling method for the determination of chlorinated paraffins
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Chlorinated Paraffins (CPs) are a complex mixture of industrial chemicals with various applications. This class of chemicals is of increasing concern due to their high production volumes, high persistence/bioaccumulation, and long range transport potential, which puts Arctic environments at risk. However, analysis of CPs is challenging due to their extreme complexity with no established method for their determination, resulting in knowledge gaps about their environmental levels and fate. The aim of this study was the development of a method for the analysis of short-chain CPs (SCCPs) and medium-chain CPs (MCCPs) in air that could be applied to assess long range transport of CPs towards Arctic regions. Using an active air sorbent based sampler previously developed, indoor air samples were collected at various sampling times (4, 8 and 22 hours) to assess optimal solvent extraction volume to elute both SCCPs and MCCPs from sampling cartridges at varying concentration levels. Once optimal extraction volume was determined, outdoor air samples were collected to evaluate repeatability and sorbent breakthrough over different sampling times (24 and 72 hours). Repeatability experiments showed concentrations obtained between parallel samplers were in good agreement for SCCPs with %RSD ranging between 1-15%. However, performance for the MCCPs was poor with %RSD between parallel samplers as high as 70%. For SCCPs, no breakthrough was observed after 72 hours collection, but for MCCPs results of breakthrough experiments were highly variable depending on the experiment. Difference in performance between SCCPs and MCCPs may be attributed to background contamination observed in the field blanks. For SCCPs, blanks were at least 10 times lower than the samples, while blank concentrations for the MCCPs were comparable or higher to concentrations observed in the samples. In addition, field blanks from the same experiments showed comparable concentrations for SCCPs, while for the MCCPs concentrations were highly variable. Blank issues surrounding the MCCPs could be due to contamination during the analytical procedure. Future work will focus on exploring sources of MCCPs contamination to improve the sampler's performace for the determination of MCCPs within air samples.

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Toxic potentials of air pollutant mixtures and the role of PAHs and their derivatives
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According to the WHO, air pollution represents a major environmental risk to human health and it has been linked to a higher incidence of pulmonary and cardiovascular diseases. Air pollution comprises a complex mixture of both known and unknown chemicals. Therefore, for the toxicity assessment, it is

convenient to employ the battery of *in vitro* bioassays which cover the complexity of such a mixture and can focus on specific molecular mechanisms of toxicity. As for potentially toxic compounds occurring in the outdoor air, polycyclic aromatic hydrocarbons (PAHs) represent an important pollutant group. Even though parental PAHs are widely studied, their derivatives like oxygenated PAHs (OPAHs) or nitrated PAHs (NPAHs), which could significantly contribute to adverse health effects, are frequently ignored. This study aimed to determine levels of 58 PAHs, OPAHs and NPAHs in ambient air as well as to describe several toxic potentials of air pollutant mixtures. Moreover, all results were further investigated with respect to gas/PM partitioning, PM size, pollutant polarity, geographical and seasonal variability and bioavailability from PM. For the purpose of this study, air samples from a heavily polluted urban site and from a background location in the Czech Republic were collected. To assess the more specific distribution of toxic potentials, gas phase, coarse particulate phase, and six PM size sub-fractions were sampled. Moreover, sample extracts were also fractionated according to polarity. Also, certain filter portions were extracted by simulated lung fluids to assess the bioavailability of studied compounds and toxic potentials. Human-based *in vitro* bioassays were employed to study anti-/estrogenicity, anti-/androgenicity, AhR-mediated activity, thyroid receptor-mediated activity and cytotoxicity for respiratory cells. From our results, it can be concluded that air pollutant mixtures possess endocrine disruptive potentials and significant cytotoxicity. Studied effects were observed predominantly in the finest fractions of easily inhaled PM and were mainly elicited by chemicals in the polar fraction. Moreover, significant seasonal variation was detected. Therefore, air pollutants can affect the health of the exposed population via different mechanisms while the contribution of PAHs and their derivatives can explain only part of observed effects. This research was supported by project GACR P503 16-11537S, by RECETOX research infrastructure (LM2015051) and by Max Planck Society.

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Analysis of PBDEs and legacy POPs, and PAHs in human breast milk from three Colombian cities
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Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polynuclear aromatic hydrocarbons (PAHs) are contamiants ubiquitous in the environment. All of these compound classes excluding PAHs were generated in relatively large volumes during their period of industrial production and are now subject to long term monitoring and restriction under the United Nations Stockholm Convention on persistant organic pollutants (POPs). Concentrations of seven congeners of polybrominated diphenyl ethers (PBDEs) were reported for first time from Colombia in breast milk (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183).Thirty-seven polychlorinated biphenyls (PCBs) and fourteen organochlorine pesticides (OCP) were also analyzed in 60 human breast milk samples from volunteering mothers collected in 2015 in three important cities from Colombia (Bogota, Cartagena, Medellin). Most of the investigated PBDE congeners were detectable in the breast milk, ranging in concentrations from 0.13 to 0.78 ng/g lipid wt. BDE-47 congener had the greatest concentration among the samples (Table 1). Overall mean concentrations of OCP oscillated between 0.44 to 6.2 ng/g lipid wt. trans-Chlordan and Endosulfan II were greatest concentrations among OCPs found in the matrix analyzed. On average, Cartagena was the city with the greatest exposure to PBDEs 0.894 ng/g lipid wt, followed by Bogota 0,35 ng/g lipid wt and Medellin 0,338 ng/g lipid wt. Overall mean concentrations of pesticides in the cities was strongly correlated with the mean of PBDEs and OCP concentrations, with averge exposure of 54.0, 364 and 248 ng/g lipid wt for Cartagena, Bogota and Medellin respectively. These results could be related to the city location and diet in each city. Cartagena is the only city located on the Caribbean coast, for instance, the population consume fish more often than the population of Medellin and Bogota. A current study investigating fish from the region of Cartagena may provide insight into local exposure. The results of this study suguest that the concetrations of PBDEs, legacy POP´s and PAHs from Breast Milk in Colombia were present at the concentration as low as countries as US, Japan, and Eurpean countries. This is first study in Colombia to resport different classes of PBDEs, legacy POP´s and PAHs from Breast Milk. ***Acknowledgement.*** Colciencias Grant No. 110759634967

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Poster spotlight

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The Environment as a Reactor Determining Fate and Toxicity of Nanomaterials (I)

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Nanopesticides in the Environment - Understanding their Fate, Bioavailability, Effects and Risks in Soil and Water
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The application of nanopesticides would represent the first intentional diffusive input of engineered nanoparticles into the environment. Yet, knowledge on their environmental fate, behaviour, bioavailability and effects is lacking. Thus, there is an urgent need to increase the fundamental scientific understanding of nanopesticides in the environment. This research is a priority from the perspective of ecosystem and human health and preservation of soil and water quality. On one side, formulation scientists need data how different carrier systems behave in the environment and how they can help designing better pesticide products and optimising the use of pesticides. On other side, risk assessors and regulators need this knowledge to judge between pros and contras of nanoformulated pesticides. Such research is a basis on which nanopesticide regulation and possible commercialization may later be established. This contribution will present the new ongoing research project of RECETOX MU which general goal is to increase understanding of the fate, bioavailability, effects and risks of nanopesticides in environmental matrices. Specific aims of the research are: 1) to adapt, optimize and verify current methods for the study of nanopesticide fate and effects; 2) to increase understanding of factors and mechanisms of nanopesticide fate, behaviour and bioavailability in soil and water; 3) to elucidate the impact of nanoformulation on pesticide toxicity and risks. After the introducing the project and its activities, the results of a pilot case study will be presented. In this study, chlorpyrifos and tebuconazole were added to LUFA 2.1 or LUFA 2.4 soils either as pure active ingredients or as conventional formulations or as nanoformulations (polycaprolactone and solid lipid nanoparticles). Then, the dissipation of pesticides in the soils and their bioaccumulation in earthworms and lettuce were measured over 3 months in simple microcosms. The results showed that the formulation of pesticides significantly affected their fate and bioavailability. Nanoformulation in several cases increased the persistence of the pesticides in soils and bioaccumulation in the earthworms. The uptake to plants was also changed by the nanoformulation but inconsistently (decrease also observed). Studies like this one will be needed in the future frequently to assess environmental pros and contras of the nanopesticides.

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Multigenerational exposure of Folsomia candida to copper agrochemicals: conventional and nano-pesticides
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Nanotechnology applications have been growing in agriculture mainly in the form of nanopesticides which are produced to have higher efficacy and therefore to reduce the number and amount of agrochemicals sprays/input to the environment. Copper fungicides are one of the most common pesticides used in conventional and organic agriculture with worldwide application. Already available in the USA market, Cu nanopesticides represent an intentional release of nanoparticles in the ecosystems that raise some concern about their possible impact in the environment. We tried to mimic two realistic agricultural scenarios where we can have a continuous exposure to the agrochemical with aging but also with a contamination coming in pulses. In both cases longer termed exposures are expected for soil populations and communities, and therefore multigenerational exposure and effects are important to assess when looking into a possible impact on the population level. To address this issue we studied the potential toxicity of a multigenerational exposure of copper to collembolan using a conventional pesticide and a nanopesticide, as well as the active ingredient $Cu(OH)_2$ in spiked soil. Survival and reproduction were assessed for three generations using two possible scenarios: 1) Aged soil, where the soil was spiked only at the beginning of the test and, 2) Copper spiking at the start of each cohort. After three generational exposure, the organisms were moved to uncontaminated soil for three extra generations to assess their recovery potential. Exposure to aged soils revealed an increasing tolerance across generations. In contrast, in treatments with freshly spiked soil, the collembolan populations showed ongoing sensitivity. After being moved to clean soil, exposure to copper a.i. and kocide produced prolonged

effects, with no recovery at least after 1 generation in clean soil (e.g. F3). This study emphasises the importance of multigenerational approaches to obtain relevant evaluations of environmental risk associated with chronic exposure to agrochemicals.

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Influence of dissolution on the uptake of metal nanoparticles in soil organisms Eisenia fetida

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The risk assessment of metal nanoparticles (NPs) is challenged by discriminating the uptake of true NPs from released ions in organisms. In soil, metal NPs bioavailability has mainly been attributed to dissolution processes because concentrations of metals in pore water have been correlated to metal accumulation in some soil organisms. However, currently it is still not possible to completely exclude or quantify the uptake of particulate metal. In order to investigate the role that dissolution plays in the uptake of metal NPs in earthworms in natural soil and to reveal any metal uptake in its particulate form, we performed a bioaccumulation test using bimetallic NPs, Au core-Ag shell NPs (Au@Ag-NPs) and combined exposure of Au-NPs, Ag-NPs, Ag and Au ions. The Au core of the Au@Ag-NPs does not dissolve, and would thus behave like an indicator of nano uptake when detected in the organisms because only the outer shell made of Ag would interact with the exposure media. Specimens of Eisenia fetida were exposed to 1.5 mg Au Kg^{-1} and 25 mg Ag Kg^{-1} in different forms of Au and Ag (ionic and/or particulate) in glass jars (n=5) containing natural, uncontaminated soil for 28 days. Au and Ag concentrations were quantified in all the exposure soils and in the organisms by inductively coupled plasma mass spectrometry (ICP-MS). Additionally, the earthworms tissue exposed to Au@Ag-NPs were analysed by inductively coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) allowing to quantify Au@Ag-NPs in their bimetallic particulate form. Metal analysis of earthworm tissue showed that comparison amongst Ag concentrations in the earthworms exposed to the different forms of Ag were not statistically different. However, the concentration of Au in the earthworm tissues exposed to HAuCl4 exceeded twenty times the others which were not statistically different\nreciprocally instead. The co-exposure to both metal ions led to a different uptake pattern compared to the single metal exposure, indicating that important interactions affecting uptake occur in the soil. ICP-TOFMS measurements gave evidence of uptake of the metals in their bimetallic nano form. In this study, it was confirmed that dissolution is the main factor driving the uptake of (dissolving) metal nanoparticles in earthworms. Uptake of non-dissolving particulate is much lower.

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Stable isotope tracing of silver nanoparticles in wheat

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The rapid development of new products that incorporate silver nanoparticles (Ag NPs), and the subsequent consumer use and disposal of such products, is ultimately associated with the release of Ag nanomaterial (NMs) waste into the environment. Such NMs enter natural ecosystems either by direct release or when Ag NPs discharged into wastewater streams are captured in sewage sludge, which is applied on agricultural land as a biosolid fertiliser. Recent research shows that the impact of Ag NPs on plant cell structures and their physiological and biochemical functions can differ from that induced by exposure to ionic Ag. Due to analytical limitations, such investigations commonly focus on uptake and effects at high and hence environmentally unrealistic concentrations, usually exceeding the predicted environmental levels by three or four orders of magnitude. Stable isotope tracing of NPs can overcome such limitations. This technique employs NPs labelled with a non-radioactive, but highly distinct stable isotope signature, which enables exposure studies with environmentally relevant NP concentrations. In the current investigation, 20 nm-sized citrate-capped ^{109}Ag NPs and dissolved $^{109}AgNO_3$ were added to separate batches of Lufa 2.2 soil to give a final Ag concentration of 72 μg/kg. Winter wheat (*Triticum aestivum L.*) was then grown to maturity under controlled conditions in these soils and plant tissue samples (seed, root, shoot and grain) were taken at eight different growth stages. The concentrations of the two labelled ^{109}Ag forms in the tissues were determined by MC-ICP-MS (multiple collector - inductively coupled plasma mass spectrometry). The analyses revealed that the ^{109}Ag uptake by wheat was higher for the exposure to ^{109}Ag NPs than for the $^{109}AgNO_3$ treatment. The highest ^{109}Ag concentrations were found in the roots exposed to ^{109}Ag NPs, exceeding those found in roots of plants exposed to $^{109}AgNO_3$ by a factor of 3 to 5. No adverse effects on the growth of winter wheat exposed to ^{109}Ag NPs and $^{109}AgNO_3$ at an environmentally relevant Ag concentration of 72 μg/kg were found. **Keywords:** nanoparticles, isotope tracing, wheat

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The influence of the plant rhizosphere on the availability of silver nanoparticles to wheat exposed in soil

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Silver nanoparticles (NPs) are expected to reach the soil environment through the application of sewage sludge to agricultural land and will be present predominantly in the form of Ag_2S. Although Ag_2S has often been considered biologically inert, it has been shown that Ag_2S can be taken up by plants and exert toxic effects. Growing plants may affect the behaviour and availability of NPs in soil, particularly the rhizosphere. Plant roots are known to exude organic acids which can impact the pH and organic carbon content of the soil, which in turn may affect the behaviour NPs in the soil. Currently it is not known if and how the plant rhizosphere conditions might influence the availabiltiy of Ag_2S to plants exposed in soil. The aim of this study is to investigate the influence of the plant rhizosphere on the bioavailability of three silver forms (pristine 20 nm Ag NP, Ag_2S NP and $AgNO_3$) to wheat, *Triticum aestivum*. Plants were exposed from seed for 14 days post-emergence in spiked soil. In order to understand how the plant roots may be influencing the availability of Ag in the soil the extractable Ag, pH and dissolved organic carbon (DOC) measured at distances from the plant roots, as well as the concentration in the root, after 7 and 14 days. Significantly higher Ag concentrations were observed in plant roots exposed to 20 nm Ag NP compared to Ag_2S NP and $AgNO_3$. The concentration of Ag in the DTPA extract was highest for the 20 nm Ag NP followed by $AgNO_3$ and Ag_2S NP exposures. There was equivalent accumulation of Ag in wheat exposed to Ag_2S and Ag^+, although there was almost double the amount of DTPA extractable Ag in the Ag^+ spiked soils. The extractable Ag in the Ag_2S spiked soils was consistently lower compared to the other two Ag forms and did not vary significantly with time or with proximity to the plant roots. Overall, this study showed that the extractable Ag in the soil is influenced more by the form of Ag spiked in the soil than by the proximity of the soil to the plant roots. However there was some evidence that soil pH decreased with closer proximity to the roots and there was increased extractable in the $AgNO_3$ exposure in the rhizosphere soil compared to bulk soil after 14 days. This data coupled with information on the structure and diversity of the bacterial and fungal communities provides insights into how the rhizosphere is influencing the bioavailability of different Ag forms in soils.

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Toxicokinetics of silver nanoparticles in the plant Brassica rapa

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Nanoparticles (NPs) are everywhere and by ongoing applications of AgNPs, the worries about their risks are increasing. Plants as primary producers may especially be exposed to the AgNPs through sewage sludge application onto agricultural land. The aim of this work was to determine the toxicokinetics of several forms of AgNPs in *Brassica rapa*. Lufa 2.2 soil was spiked with 10 mg Ag/kg dry soil of AgNPs 50 nm, AgNPs 3-8 nm, Ag_2S NPs 27 nm and $AgNO_3$. The soil was moistened to 60 % of the maximum water holding capacity. After one day of equilibration, plastic containers filled with 400 g moist soil for each treatment (Ag form) received 8 *Brassica rapa* seeds. After germination of 50% of the seeds, the test went on for another 42 days. At each sampling time (7, 14, 21, 28, 35 and 42 days) three replicates including 2 plants per replicate in the equator of soil were cut from the surface of the soil and the length and the weight of the plant were measured immediately. The different plant parts were separated and dried in an oven. The roots of the sampled plants were carefully washed three times with milli-Q water and dried. The dried plant samples were digested and total Ag content measured by Graphite furnace atomic absorption spectometry. A two-compartment model with two stages in the uptake phase was used to describe the uptake kinetics of Ag in the plants. The *Brassica rapa* plants started to flower usually 11 days after seeding, which is equal to day 7 in our test. So, maybe in the first stage of the growth (before flowering), plants tried to absorb nutrients from the soil as much as they can, possibly leading to a higher uptake of Ag in earlier life stages. In the two-compartment model used, the uptake phase therefore was divided into two stages. Stage I is the time up to day 7 before flowering and stage II is after flowering. The lowest uptake rate constant (K1a), the lowest transfer rate constant (Ki) and the highest elimination rate (K2a) were seen for the Ag_2S NP exposed plants before flowering. After flowering, the root uptake rate constant for AgNPs 50 nm (K1b) was 0.1, while that for other Ag forms was zero which means that the plants exposed to AgNPs 3-8 nm, Ag_2S NPs and Ag^+ stopped Ag uptake and continued to transfer the Ag to the shoot after flowering. The results showed that the distribution of the Ag taken up from the different forms of Ag was different in the exposed plants.

Advances in Soil Ecotoxicology and Risk Assessment - Impact, Ecotoxicity Tests, and Concepts for a Retrospective Environmental Risk Assessment (I)

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Ecotoxicological tools for precise assessment of phytotoxic effects after soil supplementation with sewage sludge
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Anthropogenic activities are increasing the contamination of soils with heavy metals that cannot be degraded and hence accumulate in the environment, having the potential to travel through the food chain. Simultaneously, the volumes of produced sewage sludge from wastewater treatment plants are consistently increasing throughout Europe. Their final disposal in agriculture can appear as a viable alternative solution to deal with such a problem. Nevertheless, re-use of sewage sludge can pose severe risks due to their content in contaminants including heavy metals. Hence, re-use must be precisely controlled to minimize the threat to both, the environment and human health. The main aim of the study was to compare and evaluate the sensitivity of different stress markers in plants exposed to soil supplemented with sewage sludge to develop more accurate toxicity tests which can be used across different taxonomic groups and to estimate the impact of sewage sludge land spreading on plants. Moreover, the exposure time needed to produce significant changes was also assessed in order to estimate if the selected markers were not only accurate but also quick to implement. The presented study aims to compare a wide range of potential stress markers in plants species from three taxonomic families: *brassicaceae*, *fabaceae* and *poaceae* after being exposed to soil supplemented with municipal sewage sludge. The tools used to assess the effects of sewage sludge on plants included the germination, biomass, roots length, the activity of antioxidative enzymes, the content of proteins, and chlorophyll, as well as the level of DNA damage and expression of metal chelators – metallothioneins and phytochelatins. Plants were cultivated in a growth chamber for a kinetic between 1 and 28 days of exposure. The dose of sewage sludge corresponded to the EU standards. Presented study showed that the incorporation of ecotoxicological tools in risk assessment of sewage sludge application on soils including the expression of metal chelators and level of DNA damage, allows detection of phytotoxic effects earlier (as soon as 24h of exposure) than with standard toxicity tests. Significant changes started to occur after 7 days from the initial exposure. Moreover, the influence of trace metals in sewage sludge on plants stress response has been assessed measuring the metallothionein coding gene expression in plants exposed to non-contaminated soil supplemented with municipal sewage sludge.

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Scaling in ecotoxicological assessment of biochar-amended soil: from single-species test to indoor mesocosms experiment
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Application of biochar for soil amelioration and as a carbon sequestration tool is focus of increasing interest, albeit the mechanisms behind its behaviour, ecotoxicity and fate in soil remaining poorly understood. The current study focused on integrative ecotoxicological evaluation of a representative biochar (slow pyrolysis of woodchips, 620ºC), added alone and in combination with compost and mineral fertilizer, through combining the responses of soil organisms and key processes. The study aimed at investigating the effects of the treatments on co-existing edaphic organisms in indoor small-scale terrestrial ecosystem models (STEMs), namely on *Eisenia andrei* survival and body weight, bait-lamina consumption, morphological and production traits of rapid-cycling *Brassica rapa*, and the effects of corresponding leachates on aquatic macrophyte *Lemna minor*. A preliminary screening bioassay was employed to investigate the effects of woodchip biochar on survival and weight changes in *E. andrei*. Statistically significant loss of body weight was observed, and the sub-lethal ecotoxicological parameters LOEC of 5% (w/w) and NOEC of 2% (w/w) were the foundations for the application rate choice in the subsequent mesocosms experiment. In STEMs, the soil was amended with the biochar, biochar-compost, NPK-based mineral fertilizer (0.5 t/ha), and their combinations, at agriculturally relevant application rates of 2% (w/w; equivalent to 40 t/ha). At the end of the 6-week mesocosms experiment, a leaching procedure was conducted on disturbed soil mesocosms. Low-to-no effect on *E. andrei* were revealed. There were no signs of hydric stress, nor treatment-induced effects on photosynthetic activity in plants. Statistically significant stimulation in *B. rapa* seeds number wasobserved, in the biochar-compost combined with mineral fertilizer. Bait-lamina consumption was reduced in the last 12 days in the biochar-amended soil treatment. *L. minor* fresh weight was the most sensitive endpoint, while dry-to-fresh weight ratios were elevated, as response to stress. Biological responses varied from sub-lethal to neutral and/or stimulatory, depending on the test organism, evaluated endpoints, and treatments. Overall, the study results suggest a low risk to soil biota from the application of 40 t/ha woodchip biochar, biochar-compost and their combinations with NPK-fertilizer. Nevertheless, the possibility of nutrients' and/or organic compounds' leaching requires further investigations.

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Comparative toxicity of parabens on soil species; plant and earthworm
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Parabens are added to cosmetics as well as medicines and foods. Therefore, they are likely to be exposed to the environment through use of personal care products. Although they are widely used in the world, there are few studies on soil ecotoxicity of parabens such as methyl-, ethyl-, and propyl paraben. This study assessed the soil ecotoxicity of methyl, ethyl, and propylparaben using plant and earthworm, and compared the toxicity of each paraben. Methylparaben, ethylparaben, and propylparaben were selected as test chemicals, and mung bean (*Vigna radiata*) and earthworm (*Eiseinia andrei*) were used as test species. Earthworm was exposed parabens for 7 days, and mung bean was exposed them for 14 days. Then burrowing rate and growth inhibition was measured as endpoint of each species. The results showed that as the concentration of parabens increased, the number of burrowing individuals decreased as compared to the control group. In the case of mung bean exposed to three parabens, statistically significant effects were observed on the growth measured at 14 days. Through the results of soil ecotoxicity, the toxicity values of three parabens for soil species were calculated. The toxicity of parabens on earthworms were high in order of methyl- < ethyl- < propyl paraben, whereas the effects of parabens on plants were high in the opposite order with the results of earthworm test. In this study, we have confirmed that parabens can affect different species in soil by using plants and earthworms. *This study was funded by the Korea Ministry of Environment (MOE) as the Environmental Health Action Program (1485014458), and the Graduate School of Specialization for managing information of chemical risk.*

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Characterization of an agricultural soil polluted by As and its impact on plant species diversity and health.
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Arsenic (As) contamination is a major health issue. 200 million of people in the world are exposed to Arsenic (As) contamination in drinking water. Moreover, accumulation of As by crop plants (rice, barley…) constitute also a risk of exposure to human. Phytoavailability of As and its compound varies among soils and the accumulation of As in plants can lead to toxic effects on growth and productivity. As a consequence, in complement to physicochemical analysis, the phytoavailability of As and its toxic effects need to be taken into account during environmental risk assessment. This study, integrated into the framework of the WaterJPI AgriAs project, investigated the phytoavailability, the phytoxicity of As and the plant species diversity related to the total As content and chemical forms present in an agricultural soil contaminated by the destruction of As-containing shells. The results showed that the As in soils from the site polluted by the destruction of WW1 ammunition is mainly present in the form of AsV and is phytoavailable. The leaf As content increases proportionally with the total As content of soils. According to the results obtained with the biotest using the leaf fatty acid composition of Lactuca sativa (Omega-3 Index), the highest polluted area is the most phytotoxic. Moreover, the Omega-3 Index measured on plant harvested on the site showed that the state of health of some native plant species (B. napus and an Unidentified plant species) seems to be affected on the medium polluted area. The absence of B. napus, P. major and the Unidentified species on the highly polluted area, can be explained by the higth content of As on soil. The state of the health of the only species found on the highly polluted aera (C. arvense and M. chamomilla) does not seem to be impacted by hight level of As even if they accumulate more As. It seems that these plant species set up defense mechanisms for the detoxification of As. These results could explain why no crop plant grown on the highly polluted area when the site was exploited for agriculture and also why only two plant species are found on this area. The implementation of biological tools on this site, in addition to physicochemical analysis, highlights the importance of taking into account the ecological impact in risk assessment.

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Tackling EFSA recommendations on testing for Ecological Risk Assessment

of Plant Protection Products (PPP) to in-soil organisms

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In the scientific opinion on the risk assessment of plant protection products for in-soil organisms, the Panel on Plant Protection Products of the European Food Safety Authority (EFSA PPR) identifies research gaps and makes recommendations to further improve the current Ecological Risk Assessment (ERA) scheme in-soil organisms. These are e.g. to improve testing and exposure conditions at lower tier, use of Species Sensibility Distribution curves (SSDs) as a potential approach for an intermediate tier and calibrate the assessment factors to attain the protection goal. This contribution aims at tackling these recommendations by presenting data on soil organisms and the insecticide Lorsban 480 (a.i. Chlorpyrifos) as model PPP. At lower tier assessment, EFSA PPR states that the test with predatory mite *Hypoaspis aculeifer* needs amendments regarding more realistic exposure scenarios. By comparing the reproduction of this species when exposed via soil vs. via soil+contaminated prey, it is shown that exposure via food significantly increased toxicity to Chlorpyrifos. Amending the test protocol to include exposure via food will increase its relevance in ERA. As a possible intermediate tier, the use of an SSD approach is proposed, despite the lack of standardized methods for soil species within the same organism group. We demonstrate by testing six microarthropods and six oligochaete species that SSDs are a feasible tools for in-soil ERA, and show that data obtained from tests with the standard species are not always representative for the group sensitivity. Finally, data on a higher tier Terrestrial Model Ecosystem study, using two application scenarios (single application and multiple applications) were performed. Specific effects were recorded, not only according to the traits of the species analyzed (microarthropods and earthworms) but also according to the application scenario adopted. These aspects need to be considered when aiming at a more realistic assessment and calibration of a protective risk assessment scheme for in-soil organisms.

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Toxicity of imidacloprid and thiacloprid to eight species of soil invertebrates

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Since 2013, imidacloprid has its use restricted within the European Union, due to its toxicity to bees. It is also known to be toxic to aquatic invertebrates, but there is a gap in the knowledge concerning its toxicity towards soil invertebrates. Aiming to bridge this gap, this study raised three questions: *(i)* Is there a difference in sensitivity between the taxonomic groups of Annelida and Arthropoda?; *(ii)* Is there a difference in toxicity towards the different endpoints measured (survival and reproduction)? *(iii)* Is there a difference in the toxicity towards different modes of reproduction? Ecotoxicological tests were performed to assess the toxicity of imidacloprid and thiacloprid to *Folsomia candida, Sinella curviseta, Folsomia fimetaria, Heteromurus nitidus, Enchytraeus crypticus, Eisenia andrei*, *Porcellio scaber* and *Oppia nitens*, in Lufa 2.2 soil. Imidacloprid was the most toxic neonicotinoid to all the species tested and to both endpoints. There were no differences in toxicity between the taxonomic groups (Annelids, Arthropods) nor within the different modes of reproduction (parthenogenetic versus sexual), with sensitivity being species specific. The most and least sensitive species to both compounds were arthropods. Within the Annelida, sensitivity of *E. andrei* was more similar to that of the springtails than to *E. crypticus*. It is known for insects that the difference in sensitivity between neonicotinoids can be related to the concentration of different subunits present on the nicotinic acetylcholine receptors. A high concentration of α subunits (high imidacloprid affinity) can indicate a high sensitivity of the species to this compound, and so on (Thany et al. 2006; Tomizawa and Casida 2003). It remains unclear which subunits are present in the species tested. Based on recommended apllication rates, we calculated the PEC of imidacloprid to be 0.03 mg/kg in the top 5 cm soil layer. In earlier laboratory toxicity tests we found a DT_{50} of ~125 days (Lufa 2.2 soil). The application interval sugested for the formulation Confidor is ~7 days, so after 8 applications in one year, the concentration could increase up to ~0.13 mg/kg. This means that the PEC is already within the range of the EC_{10} valuesand could eventually cause some effect on the reproduction of *F. candida*, *S. curviseta* and *F. fimetaria* (0.09-0.12 mg/kg dry soil) or affect the ecosystem services these species provide, such as the decomposition of organic matter.

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Do we use plant protection products correctly? Effect of insecticides on the development of the solitary bee Osmia bicornis

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Solitary bees provide essential pollination services for many arable crops, but are prone to global decline due to intensive agricultural practices such as pesticide usage, posing a major threat to the food security and ecosystem stability. Understanding pesticide toxicity to bees is crucial for protecting pollinator populations, as it may not be possible to cease pesticide usage at the moment because of the growing demand for food. The solitary red mason bee *Osmia bicornis* is regarded as a model organism for pesticide risk assessment but the scarcity of information about effects of pesticides to this species limits its use in pesticide regulation scheme. In this study, the effect of three commonly used insecticide formulations on the development of *O. bicornis* was studied by exposing larvae to insecticide-contaminated multifloral pollen. The insecticides were: Dursban 480 EC, containing the organophosphate insecticide chlorpyrifos (CHP), Sherpa 100 EC, containing the pyrethroid cypermethrin (CYP), and Mospilan 20 SP, containing the neonicotinoid acetamiprid (ACT). At all five tested concentrations of the formulations containing CHP and CYP, significantly lower larval survival was observed with respect to the control ($P \leq 0.01$). Significant decrease in the 18-day larval body mass ($P \leq 0.003$) and cocoon weight ($P \leq 0.02$) further supported the severe influence of CHP and CYP formulations on *O. bicornis* development. Moreover, a significant delay in cocoon formation was observed for CHP-treated larvae ($P=0.008$) but not in the larvae exposed to CYP ($P=0.4$). In contrast, formulation containing ACT did not affect larval survival ($P=0.90$). Also, neither larval body mass nor cocoon weight ($P=0.4$) was affected by ACT and only marginally significant delay in cocoon formation ($P=0.08$) was found. Although the effect found so far in ACT-treated larvae was moderate, the emergence rate of F1 progeny and their later survival rates are still in question. Overall, it is clear from this study that at least some insecticide formulations, here those containing CHP and CYP, affect the development of *O. bicornis* even at concentrations actually found in pollen in the field, indicating an urgent need for revising current pesticide usage recommendations. This study was supported by National Science Centre Poland (grant No. 2015/19/B/NZ8/01939).

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Development of a test method for herbivorous lepidopteran larvae

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Currently, the environmental risk assessment of Plant Protection Products (PPP) does not consider the exposure via food ingestion of herbivorous arthropods. Therefore, a prominent route of exposure is not addressed in standard test protocols. To help filling this methodological gap, the testing of lepidopteran larvae was proposed by the EFSA (2015) to represent herbivorous non-target arthropods in eco-toxicological testing, as they play an important role in the ecosystem and are widely distributed across Europe. This study aims to contribute to the environmental risk assessment of PPP, by developing a new test method suitable to assess the potential effects of oral exposure on herbivorous lepidopteran larvae. The development of the test method was divided in three major steps to examine the suitability of the chosen method: 1) the rearing of the species *Vanessa cardui* and *Pieris brassicae* under controlled laboratory conditions 2) the test system development with the key aspects: route of exposure, environmental test conditions, test units, life stage, synchronization, reliability and reproducibility 3) the most relevant and suitable endpoints (e.g. mortality, feeding rate, pupation rate) for the test system considering the mode of action of the pesticide applied. Furthermore, the shortcomings, recommendations and further improvements of the test method will be discussed.

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Multi-stress effects on honey bee colonies

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High losses of honey bee colonies in recent decades are of great societal and economical concern and has been experienced as sign of vulnerability of agriculture, including the service of crop pollination, and of beekeeping. *Varroa destructor* infestation is acknowledged as an important cause of these losses and often suggested to act in concert with contributing stressors, such as or exposure to insecticides, infestation by *Nosema spp.,* or low or monotonous food availability. In several different field experiments, we studied the relative and interactive effects of *V. destructor* infestation and these stressors at field-realistic exposures on the performance and survival of honey bee colonies. Colonies infested by *V. destructor* were 13% smaller in size and were 59.1 times more likely to die than colonies infested with low levels of *V. destructor*, but in contrast to the expectations no interactions with *Nosema spp.* or imidacloprid were found for colony size or survival. Nor did we find a direct effect of imidacloprid on colony size or survival. At individual level in these colonies however, pollen foragers from colonies exposed to *V. destructor* in combination with imidacloprid flew less far (when tethered in a flight mill) compared to colonies exposed to a single stressor. Colonies as a superorganism may well be able to compensate at the colony level for negative effects of stressors on their individuals. In all of our experimental studies under field realistic exposure to stressors, *V. destructor* was by far the most lethal stressor for honeybee colonies.

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BEEHAVE validation and resulting insights for the design of field studies with bees
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Factors affecting honey bee health are manifold (including diseases, parasites, pesticides, environment and socio economic factors). A lack of standard procedures for higher tier risk assessment of plant protection products for bees and the inability to conduct higher tier studies for all plant protection products, their different use patterns, and at different climatic zones makes coherent availability of data and their interpretation and the use for higher tier risk assessment challenging. Focus has therefore been given to the development of modelling approaches which in the future could fill this gap. BEEHAVE is the first model attempting to link two of the processes vital for the assessment of bee mortality; the within-hive dynamics for honey bee colonies and bee foraging in heterogeneous and dynamic landscapes. Here we show results of several BEEHAVE validation studies conducted. We specifically focusing on insights gathered through these modelling exercises for the design and the usability of field studies for further development, testing and validation of the BEEHAVE model. Overall the model validation shows that predictions of bee hive dynamics fit observations of the total number of adult bees, the total number of offspring in the hive, and the production of drones well. This result underpins the results of the EFSA evaluation of the BEEHAVE model that the most important inhive dynamics are represented and correctly implemented in the model with empirical evidence. Agreement between data and model predictions is particularly high for the initial experimental phase prior the generally conducted relocation of the bee hive from the actual experimental landscape to an overwintering site. Increased discrepancy following the relocation is an artefact of lack of information on the landscape characterisation of the overwintering site for model parameterisation; leading to increased inaccuracy of the model prediction for pollen and nectar resources in the hive, that in turn determines the abundance of bees and thus the overwintering survival probability of the colony. To better assess the suitability of using BEEHAVE for the prediction of bee colony overwintering survival as an important endpoint for higher tier risk assessment for bees it is vital to redistribute experimental efforts allocated to a field study towards a more equal bee hive and landscape investigation throughout the entire experiment, rather than a bias towards the actual exposure phase.

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Modelling the impact of insecticides on arthropod populations of agricultural interest
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Mortality and median lethal dose/concentration (LD50/LC50) are used as toxicological endpoints in 95% of published studies in the SELCTV database. Such methods do not provide enough information about the actual effects that may occur in pesticide-exposed populations over periods longer than a few days, resulting in an oversimplification of the potential consequences of pesticide-induced stress. A demographic approach provides better toxicological information than lethal dose estimates We present two case studies where Matrix Population Models (MPMs) are used for assessing the potential impact of insecticides on a beneficial insect - *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) - in laboratory and for evaluating the resurgence of the two-spotted spider mite - *Teranychus urticae* Koch (Acarina: Tetranychidae) after insecticide application in semi-field conditions. The general model used to simulate population dynamics consists of a matrix containing *i*) survival probabilities and *ii*) fecundities of the population. In both case studies, demography has been used to evaluate the effects of pesticides on populations by means of a Life Table Response Experiments (LTREs). In the first case study exposure of adult stage to fresh residues on leaf surface of rynaxypyr, spirotetramat flonicamid and spinetoram significantly reduced population growth index (λ) in comparison with control. Direct exposure of *A. bipunctata* adults to imidacloprid also produced a significant reduction of λ. LTREs analyses showed very different effects on the vital rates among insecticides. In the second case study the estimated matrices supported the hypothesis that some insecticides, namely ethofenprox, deltamethrin and beta-cyfluthrin, fostered a higher mite population growth than the untreated control. LTREs indicated that the higer population growth was mostly due to increased adult fecundity and egg fertility, thus suggesting a likely trophobiotic effect. Demographic approach showed to be effective to evaluate both lethal and sub-lethal effect of the insecticides either on beneficials and pests. Moreover, LTRE resulted a very sensitive method and pointed out significant differences among insecticides belonging to the same group or sharing the same mode of action. Moreover, we demonstrated that the demographic approach can be extended to semi-field and field studies for the assessment of pesticide effects. Keywords: Matrix Population Models, Ecological risk assessment, Sublethal effects, Spider mites resurgence.

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Assessment of toxic reference chemicals of honeybee brood studies under semi-field conditions in Korea
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Honeybee semi-field tests based on OECD No. 75 were successfully perfomed for 3 years in Korea. Honeybee semi-field tests were carried out to confirm the sensitivity of the method using two reference chemicals (dimethoate and diflubenzuron) in 2016 and to assess the effect of carbaryl wettable powder (WP) and imidacloprid microemulsion (ME) from April to May in 2017 and 2018, respectively. The experiments included three treatment groups (control, toxic reference chemicals (dimethoate and diflubenzuron), and test materials (carbaryl WP and imidacloprid ME), each with three replicate tunnels. The honey bee colonies were introduced in the tunnels with a size of $70m^2$ containing flowering *Brassica napus*. The dimethoate emulsifiable concentrate (EC) 46% and diflubenzuron wettable powder (WP) 25% were used as reference chemicals. The toxic reference treatments were 400 g dimethoate a.i./ha and 600 g, 800g diflubenzuron a.i./ha. The mortality of the honey bees, foraging activity, brood development, and condition of colonies were assessed during the 28 day testing period following BFD 0 (brood area fixing day 0). For the brood assessment, 200 cells containing eggs were selected and evaluated by the computer assisted digital image analysis. The mean brood termination rates (BTRs) ranged from 20.50 to 31.33% in the control group. Treatment with dimethoate and diflubenzuron led to a drastic decrease in the brood development, resulting in BTRs ranging from 68.0 to 100.0% .

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Combination of six different zebrafish embryo behaviour assays for neurotoxicity screening
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In the last decade various behaviour assays using zebrafish embryos and larvae were developed, aiming for a fast and easy to use screening tool able to detect neurotoxic substances. Tests like the light/dark transition test, locomotion test or photo motor response (PMR) test were successfully applied by different scientists to identify compounds with potential neurotoxic effects. So far, most studies perform only single behaviour tests a combination of different assays is rarely done.Therefore, this study combined six different zebrafish embryo behaviour assays to screen for neurotoxic effects of 10 pesticides, known to affect organisms, and one spiked environmental extract. The pesticides were tested in four different concentrations in a continuous exposure (until 0-5 days post fertilisation) and the applied behaviour assays were: Spontaneous movement, PMR, locomotion, touch response, thigmotaxis and light/dark transition. The behavioural tests are further validated using biomolecular techniques like the AChE inhibition measurments, transcriptomics and metabolomics.Our aim was to assess the applicability and usefulness/significance of combining all these assays for medium-high through put neurotoxicity screening.Although the PMR is easy to perform its results showed different and inconsistent outcomes, leading to the conclusion that this assay needs further optimisation. The touch response assay was less sensitive than the other tests possibly due to the big variation in behaviour after the touch stimulus. But even with this test embryos with strong effects could be identified. The commonly used tests such as light/dark transition and locomotion were more reproducible and sensitive and seem to be better suited to identify neurotoxic compounds. With the combination of tests more compounds were classified as potentially neurotoxic than when using only single behavioural test. This shows that a combination of tests might be better suited for neurotoxicity screening than single tests. To further improve the use of such a screening approach different exposure times will be investigated to account for the influence of the onset of metabolism on neurotoxic effects. Also, including further tests such as avoidance behaviour could be helpful tools for screening and are therefore considered for further investigation.

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FISH EXTENDED ONE GENERATION REPRODUCTION TEST: A COMPARISON BETWEEN MEDAKA AND FATEAD MINNOW
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The Medaka Extended One Generation Reproduction Test (MEOGRT) is a standard OECD Test Guideline (No. 240) using multiple generations of fish to generate data relevant to ecological hazard and risk assessment of chemicals. The test evaluates population relevant parameters, such as survival, gross development, growth and reproduction but also includes endpoints which may

provide evidence of endocrine disrupting properties of a chemical such as measuring vitellogenin (Vtg), phenotypic secondary sex characteristics (SSC) and histopathology. The MEOGRT has been designed and validated for use with the Japanese medaka (*Oryzias latipes*). The small size, relatively short life-cycle, easily identifiable phenotypic secondary sex characteristics and genetic sex determination makes *O. latipes* a suitable species for laboratory testing. However, for some potential endocrine affecting chemicals; this may not be the most sensitive, and hence appropriate, species to use for testing. How then can the methodology be best adapted for use with different species and what are the challenges posed? This presentation compares experimental control data for *O. latipes* and fathead minnow (*Pimephales promelas*) showing how the methodology was adapted to achieve a successful outcome and ensure regulatory acceptance and the pros and cons for the use of each species.

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Advancing the zebrafish embryo test for endocrine disruptor screening

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Endocrine disrupting chemicals (EDCs) have been receiving increasing attention in chemical regulations in the European Union. New and improved approaches are needed to meet evolving regulatory requirements worldwide, while at the same time reducing the use of laboratory animals. In this context, the zebrafish embryo offers particular advantages. In this study we provide additional tools and methods to advance the use of the zebrafish embryo for obtaining the required information on ED properties: (1) we explored the use of micro-injection into the yolk of zebrafish embryos as a way to overcome the difficulties related to aquatic exposure to EDCs, which are mostly hydrophobic, (2) we also propose additional endpoints to inform on the ED mode of action. We first compared micro-injection to the traditional aquatic exposure route using five pharmaceuticals with different ED modes of action and observed similarities but also important differences in the responses. We studied these differences in more detail and first focused on toxicokinetics. In both exposure scenarios, internal doses of 17α-ethinyl estradiol (EE2) decreased after 24 hours post fertilization while this was primarily expected after injection, showing the importance of biotransformation and/or elimination in both exposure routes. Next, we observed comparable estrogen receptor (ER) activation after aquatic exposure and injection using a transgenic reporter line, and brain aromatase and vitellogenin (vtg) mRNA levels responded in both scenarios. While vtg protein levels are widely used as a biomarker in adult fish, in embryo-larval stages vtg has so far only been shown to respond to ED at the transcriptional level. Vtg protein levels responded to strong (EE2), moderate (4-tert-octylphenol) and weak (bisphenol A) ER agonists after aquatic exposure. The lower sensitivity of this endpoint seemed to limit the potential to detect vtg alterations after injection. We also observed an increased number of functional hair cells in the developing neuromatsts after exposure to 10 ng/L EE2. While the differences in toxicity between aquatic exposure and micro-injection were mainly observed at high levels of biological organisation, low level markers of ED responded in both exposure routes. Micro-injection could therefore be used for screening hydrophobic chemicals. New endpoints such as vtg protein levels and neuromast development can be used to provide additional information on the ED mode of action.

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Assessment of the effects of binary mixtures of PAHs on the cardiac function of the rainbow trout (Oncorhynchus mykiss) larva

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Fish early life stages (ELS) are among the most sensitive to the developmental toxicity caused by polycyclic aromatic hydrocarbons (PAHs). In addition to the well-documented aryl hydrocarbon receptor (AhR) mediated toxicity of some PAHs such as retene, some weaker AhR agonists like phenanthrene can also produce cardiovascular defects such as arrhythmia via AhR-independent mechanisms. The aim of the present study was to assess the effects of several PAHs, alone or in mixture, on the cardiac function of the rainbow trout (*Oncorhynchus mykiss*) ELS. Newly hatched rainbow trout larvae were exposed for 1, 3 or 7 days to three different individual PAHs, retene (RET), pyrene (PYR) or fluoranthene (FLU), at sublethal doses (3.2, 10 and 32 μg/L for RET and PYR; 5, 50 and 500 μg/L for FLU). Binary exposures to either RET or PYR in combination with FLU were also performed for all tested doses. For each larva, the heart rate (BPM) and the average standard deviation (SD) of the interval (seconds) between each heartbeat were measured. This later parameter was used as an index of the heartbeat regularity. Larvae were scored for deformities (oedemas, haemorrhage and craniofacial deformities) and morphometric parameters such as larval length and yolk sac area were also measured. The heart rate was significantly reduced already after 1 day of exposure at the highest dose of FLU, alone or in combination with the highest dose of PYR. However, the heartbeat regularity was unaffected after 1 day of exposure at any dose and for any compound, alone or in mixture. After 3 and 7 days, the heart rate was significantly reduced at the highest dose of FLU, alone or with any PYR dose. The intermediate dose for FLU also significantly reduced the heart rate at 3 and 7 days, but this effect was almost entirely abolished when combined with the low or medium doses of PYR, suggesting possible non-additive or antagonistic interactions between the two compounds. Heartbeat regularity was significantly altered after 3 and 7 days at the highest dose of FLU, alone or coupled with any PYR dose: larvae exposed to those treatments displayed very clear signs of arrhythmia. The data from the exposure to RET alone or in combination with FLU is still under analysis. The assessment of the expression of several genes (including *cyp1a*) via qPCR in individual larvae is underway, as well as exposures involving binary mixtures of phenanthrene and fluoranthene.

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Carcinogen Induced High-Throughput Screening Model of Pro-Angiogenesis in Zebrafish Embryo-Larvae

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Tumour angiogenesis is a key target area in cancer drug development. Evaluating the efficacy of novel angiogenic inhibitors require development of suitable animal models in which vasculature can be easily explored. Zebrafish, a vertebrate organism has proved to be a promising model in cancer research. The optical transparency, highly characteristic blood-vessel patterning with short period of development (96 hour post fertilization; hpf) make the zebrafish embryo-larvae best suited for vascular biology studies. Normal vasculature of zebrafish embryo-larvae has been exploited to screen anti-angiogenic compounds. Compared to normal vasculature, tumour induced vasculature shows profound morpho-functional modifications. Accurate efficacy of a drug can be reached by studying its effect on tumour angiogenic or pro-angiogenic model. Engrafting tumour cells into zebrafish embryo-larvae (tumour xenograft) is a widely used technique to trigger angiogenesis. It requires fluorescently labeled human cancer cells, skilled personnel and huge number of tumour xenografts for large scale screening. Treatment with carcinogenic chemical can be an easy and rapid alternate approach to induce pro-angiogenesis. In this study, we used known carcinogenic chemicals, lindane (an organochlorine pesticide) and benzo-a-pyrene (polycyclic aromatic hydrocarbon pollutant). Zebrafish embryos (24 hpf) were exposed to four sub-lethal concentrations for each of the two compounds along with appropriate control. At 96 hpf, the larvae were subjected to whole mount alkaline phosphatase staining allowing microscopic evaluation of two angiogenic parameters [number of sub-intestinal vessels (SIVs) and vessel length]. As compared to the development of normal angiogenic basket formed in the dorsal-lateral part of the yolk in the control larvae, benzo-a-pyrene and lindane exhibited pro-angiogenesis which was evident by their potential to disrupt the angiogenic basket giving rise to outward and internal branching of the vessels. This zebrafish efficacy-toxicity model of pro-angiogenesis can be employed as a high throughput screening tool for dual purpose. Firstly, to screen for pro-angiogenic / carcinogenic potential of compounds, resulting in selection of safer compounds entering the agricultural market as well as cancer risk assessment of harmful pollutants. Secondly, the carcinogen induced pro-angiogenic model can be used to screen for potential anti-angiogenic compounds in cancer drug discovery.

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CHARACTERISATION AND 3D RECONSTRUCTION OF MELANOMACROPHAGE CENTERS IN SHORTHORN SCULPINS (Myoxocephalus scorpius)

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Melanomacrophage centres (MMCs) are distinct aggregations of pigment-containing cells in internal organs of fish, amphibians and reptiles. Although MMCs are commonly used as biomarkers for anthropogenic exposure in many environmental monitoring programs, a substantial knowledge on characteristics of MMCs is required prior to assess MMC responses. The present study is the first to reconstruct the 3D structure of splenic MMCs of a fish from a number of consecutive sections using the Fiji and AutoCad software. Most splenic MMCs of shorthorn sculpins (*Myoxocephalus scorpius*) had spherical shape and limited variation in size (maximum diameter). We confirmed the close relationship between MMCs and blood vessels in spleen of shorthorn sculpins as 97% of investigated MMCs (60 whole MMCs over 510μm thick of the samples) were closely associated with splenic blood capillaries (mainly ellipsoids) at least once in a set of consecutive sections. It was obvious that the bigger the MMC (maximum diameter) the more likely it would be associated with more and larger blood vessels. In this paper, we provide evidence for the presence and dominance of pheomelamin in MMCs of shorthorn sculpins.

Bio-Based Industries: Sustainability Benefits of Technological Innovation and Closed Loop Approaches across Supply Chains

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Recycling of biowaste: LCA methodology for different purposes
K. Lyng, Ostfold Research
The circular economy package adopted by the European Union introduces ambitious goals of increasing the amount of material sent to recycling. As the share of source separated biowaste increases, new treatment routes will be formed. To obtain development of sustainable value chains and to ensure reduction of environmental impacts, policy makers and decision makers need information about the environmental impacts of different choices. A comment treatment method of food waste from households and industry is anaerobic digestion, which generates two separate products: biogas and digestate. While biogas may be used to generate heat and electricity or as a fuel for transport if the methane share is increased, digestate can be used as biofertilizer or as compost/soil amendment product. Biogas used as a fuel for transport and digestate used as biofertilizer is identified as the applications that will lead to the largest reduction of environmental impacts in Norway. This application does, however, appear to be the least profitable option for the large scale biogas plants. To obtain the largest reduction of environmental impacts, there is a need for documenting the strengths and weaknesses of the end products, biogas and biofertiliser, in a life cycle perspective. This can stregnthen the market position of the most sustainable products by enableling procurers to perform green procurement by using environmental impact as a criterion when purchasing fuel for transport and fertilisers. Life cycle assessment can be used to evaluate the environmental impacts of products and services in a value chain perspective and can give input to decisions in different contexts. In this study we compare LCA methdology for two purposes for biogas value chains: input to policy development and the use of environmental product declaration in public procurement. The results show major differences in LCA methody for two different purposes.

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Sustainability check for bio-based technologies: a LCA review
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Industries across the world are making a transition to renewable feedstock for the production of organic chemicals, fuels, and materials, as new technologies based on biological principles are becoming increasingly available. However, large-scale bio-based production is not necessarily sustainable if relying solely on traditional biomass sources. Key Enabling Technologies (KETs) are frequently seen as a viable solution to increase resource efficiency and deliver further environmental and economic gains. A systematic review on life cycle assessment (LCA), Life Cycle Costing (LCC) and technoeconomic analysis (TEA) of KETs has been carried out, in order to identify potential impacts from emerging bio-based technologies that foster biomass use and processing. We depart from our own classification developed based on feedstock and, alternatively, on technology, as follows: F1) Crop residues and perennial plants, F2) Designer crops for optimized biomass content, F3) Algae based biomass, FC) Waste or recycled material, C1) Enzyme/microbial assisted processing, C2) Biorefineries. From the systematic search, 31, 2, 13, 33, 46 and 41 publications were obtained for F1, F2, F3, FC, C1 and C2, respectively. Most of them focus on the environmental dimension, showing that there is potential for climate change and non-renewable energy use savings in F1, F3 and C2, as compared with fossil energy carriers, mainly due to co-product credits. Implementing material uses towards integrated biorefineries delivers further gains in F1-F3, although results in terms of other impacts are ambiguous or disadvantageous. Our study shows that the most effective option to improve the environmental performance of bio-based systems is to employ F3 feedstock with C2 conversion technologies, especially in cradle-to-grave or cradle-to-cradle designs. Further harmonization in cut-off criteria for defining the system boundaries is desirable to enhance comparison between KETs in different technological and political contexts. This systematic assessment provides insights on already feasible technological applications for advanced biomass uses, while highlighting options for improvement in the often overestimated sustainability performance.

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Evaluating the environmental performance of facilitated synergies for producing soil and surfaces through industrial symbiosis
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Industrial symbiosis (IS), where different entities collaborate over energy, utilities, materials or services, has been identified as an approach to improve resource efficiency and circularity for industry. This article reviews the environmental performance of an IS network with firms involved in waste management, soil, surfaces, paper, lumber and energy. The aim of this study is to highlight the environmental performance of an IS network and pay particular attention to the value of facilitation services for the firms of the IS network and their products. This is done through the use of life cycle assessment to review the current IS network and compare with a reference scenario and a potential future development. The results suggest that there are significant benefits from the IS network. Large reductions in greenhouse gas (GHG) emissions and local impacts, namely eutrophication and acidification impacts are illustrated. Furthermore, large reductions in abiotic resource depletion were also identified. It was shown that all firms in the network benefit from the synergies involved, with a large share of the benefits stemming from the facilitated exchanges with the waste management company. The replacement of conventional products and energy streams with bio-based counterparts from within the network is of significant importance. Finally, the results point to the importance of the facilitation of by-products and wastes, and the significant value this creates in the region, with large potential to improve the environmental performance of firms and their products.

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Building Product Fossil, Recycled and Renewable Polymers
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Ecologically sustainable development goals are driving technology change and increasing reliance on economical, equitable, recyclable and renewable supply chains for circular economies. Key environmental pressures include global scale climate change, litter killing wildlife and pollution contaminating supply chains. In response, industry innovation and technological transformations are enabling conversion of traditional fossil, recyclable and renewable energy and feedstocks into improved and new products. Normal post-consumer (PC) recycled and biomass plastics are now in increasingly high demand. This study compares life cycle inventory, impact and benefit assessment of fossil Polyethylene Terephthalate (PET), PC recycled PET, and corn-based polyactide (PLA) used in building products e.g. insulation, reinforcing fibres, fitout materials and fabrics for production systems in America, Europe and the Pacific Rim. It compares water, feedstock and energy use outcomes of competing recycled, renewable and fossil feedstock cohorts. Land-use change and ensuing global warming potential of emissions are also discussed. The study reviews the authors' cradle to grave case studies of Pacific Rim sourced PC recycled PET building products, NatureWorks study of PLA and PET background data from PlasticsEurope. All data are from primary producers and manufacturers. Although PLA may be the preferable option for some (building) products, there are certain properties that may limit its use in certain building applications such as in-wall insulation. PLA's biodegradable and compostable properties make it less suitable for long-term used products as in-wall insulation products. PLA was also found less suitable for packaging of insulation material than PET, as the tensile strength of PLA is lower. The pigmentation use and percentage of renewable energy in the grid can be of great influence on the Life Cycle Impacts of the three studied materials.

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EPDs for Wooden Average Data Construction Products
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The EN 15804:2012+A1:2013 compatible Environmental Product Declarations (EPDs) have been gradually introduced in several countries, in Finland since 2016, ensuring a platform for commensurable assessment of construction products. In this paper we discuss on problems that arise concerning wooden average data construction products. They are especially useful for the micro and small enterprises, to whom the value of own EPD is beyond economy. In our case, the first example is the laminated log wall structure and the second case is LÄHIPUU® (Local Wood) lumber. According the standard, average data shall be derived from specific production processes. The EPD is calculated using producer weighted averages. However, there are no clear rules how the variation due to the sample should be communicated. In our case we have chosen the standard error of the mean (SE). The practicality of SE is that the 95 % confidence interval for the estimated impacts is roughly within two times the SE. EN 15804 compatible EPD are based on modules. The minimum requirement are A1-A3 (product stages), A4-A5 (construction) and C3-C4 (EoL). Additional benefits can be reported in module D. It is also possible to include carbon stock in the module A1-A3, but its treatment (release) should be included in C3-C4. Substitution gains from energy use of demolished wood can be reported in module D. The declared unit for the cases 1 m^3 of laminated log and 1 m^3 sawn and dried lumber. Altogether 5 log house factories were inventoried, presenting production of 16 members of the Finnish Log house Industry Association. LÄHIPUU® is the trademark of Finnish Small Sawmills Association with approximately 240 members. Three production alternatives were modelled. Tractor powered mobile sawmill (9 members), and fixed sawmills with sun dried lumber (4 members) and 5 members with kiln drying facility. For the time being, specific data of the Finnish modern forest management and harvesting is not available, but the standard allows generic data to be used for A1, albeit it can be an important source of uncertainty. The generic data set for roundwood induces 12% to 48% of the EPD's impacts. As the present data set is valid but rough, urgent revision is needed to ensure credibility of Nordic wood product EPDs. Variation between average product's producers

appears not to be that large as one could have *ex ante* expected. Special effort should be put on how to communicate on avoided GHG emissions and carbon stock

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Poster spotlight

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Understanding the Complexity of the Natural Aquatic Environment Facing Global Changes

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Influence of water flow and light availability on intracellular biofilm geosmin formation
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Global climate models have predicted that changes in mean values and increased variance in amplitude of climatic parameters will inevitably lead to more frequent and more intense extreme events. Alterations in flow regime are expected to severely affect freshwater ecosystems in several regions e.g. the Mediterranean. In addition to environmental factors, flow can also be affected by anthropogenic factors, such as dams. Human activities can also affect river banks causing riparian forest modifications and consequentially altering the quantity and quality of light potentially reaching riverbed. Environmental or anthropogenic variation of light and/or flow regimes accounts for much of the variation in the physiology, population growth, community structure and functioning of benthic microbial communities. Some of these alterations are associated with the appearance of tasty and odorous compounds in river waters. Among others, geosmin is a metabolite produced mainly by cyanobacteria associated to benthic biofilms. The environmental and anthropogenic factors associated with its production are still not clear. For this reason, geosmin episodes, in freshwater ecosystems exploited for drinking purposes, still suppose an economic problem for the water supply companies since they cannot predict its appearance. This study aimed to evaluate, under controlled conditions, the effects of water flow and light availability on biofilm structural and functional development, and intracellular geosmin occurrence. We performed a 6 weeks mesocosm experiment in late winter – early spring. The mesocosms were a set of 10 outdoor 3m long flumes receiving water continuously from the Ter River (Catalonia, NE Spain). Five gradually increasing flows from 0.02 to 0.16 m/s were set and combined with two light intensities: full natural light and reduced by 80%. Water samples were taken to analyse nutrients uptake. Biofilm samples were taken to analyse geosmin concentration, chlorophyll a content and the community. The results obtained shows how the nutrients uptake varies depending on flow and irradiance. It has also been shown that the interaction of lower velocities with low irradiance favours the growth of benthic *Oscillatoria* sp., and that under these conditions it can produce geosmin, a metabolite that once released into the water generates a bad taste and odour to it provoking serious issues to drinking water companies and consumers.

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Integrating daily temperature variation and life stages when assessing the reciprocal impact of sensitivities to a pesticide and to climate change
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Because of failing risk assessment and global climate change there is increasing attention for the reciprocal effects between climate change and pollutants to improve risk assessment. Two important and connected topics have been largely ignored in this context: (i) the role of daily temperature variation (DTV) as a key component of climate change, and (ii) differences in sensitivity to climate change and pollutants between larvae and adults. We investigated whether DTV magnified the negative effects of the organophosphate pesticide chlorpyrifos (CPF) on mortality and heat tolerance and whether this effect was stronger in aquatic larvae than in terrestrial adults of the vector mosquito *Culex pipiens*. Exposure to CPF imposed mortality and reduced the heat tolerance (measured as CTmax) in both life stages, which is consistent with the TICS concept ("toxicant-induced climate change sensitivity"). Notably, larvae were more sensitive to CPF than adults both in terms of mortality and in reduction of heat tolerance (CTmax), highlighting the life-stage specificity of the TICS concept. While DTV had no direct negative effects, it magnified the toxicity of the pesticide in terms of larval mortality, which is consistent with the CITS concept ("climate-induced toxicant sensitivity"). Moreover, DTV caused a stronger CPF-induced reduction in heat tolerance of adult females, thereby illustrating the coupling of both concepts and providing support for the reciprocal effects between climate change (here DTV) and pollutants. Taken together, our results highlight the importance of integrating DTV and life-stage specificity to improve risk assessment of pollutants under climate change.

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Does water scarcity influence the impact of anthropogenic stress on aquatic invertebrate assemblages?
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Combined effects of chemical pollution and water scarcity are one of the most important threats to aquatic ecosystems in (semi-)arid regions of the world. The low dilution potential in aquatic ecosystems receiving wastewaters can enhance chemical exposure, leading to devastating direct and indirect toxic effects. The main objective of this study was to assess the combined effects of pollutants related to anthropogenic impact on aquatic ecosystems in scenarios of water scarcity. In particular, we evaluated the response of aquatic invertebrates in the semi-arid region of Madrid (Central Spain) based on the taxonomic and functional variation of communities exposed to chemical pollution associated to anthropogenic impact, and additionally, to water scarcity. Twelve sites were selected in the watershed of the Tagus river and sampled at three seasons (spring, summer, fall) covering a gradient of anthropogenic impact and hydrological stress. Water quality was assessed by measuring physico-chemical parameters (pH, T, conductivity, DO, DOC, nutrients), metals and organic microcontaminants in water. At each site, the macroinvertebrate community was sampled with a Surber net in triplicate along a river transect. We used univariate and multivariate statistical techniques to evaluate how anthtopogenic and hydrological stressors may affect the invertebrate communities, and the relative utilization of biological traits. Environmental variables did not show clear seasonal patterns, but significant differences were identified between groups of sites with different anthropogenic impact and hydrological condition. Highly impacted sites with low hydrological stress were characterized by high TUs, nitrites and phosphate whereas highly impacted sites with high hydrological stress had also high conductivity and suspended solid fluctuations. Moreover, despite that the environmental data did not show a clear seasonality, biological responses acted as an integrative indicator of anthropogenic and hydrological stress (mostly in summer and early fall) in terms of taxonomic and functional composition. Hydrological stress mainly influenced reproduction and feeding types. This study proves that hydrological and anthropogenic stress interact to affect macroinvertebrate communities. The interaction between these stressors should be taken into account in further updates on the ecological status assessment of surface waters.

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Effects of S-metolachlor and terbuthylazine in the biochemical profile of the estuarine species Scrobicularia plana
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This study highlights the impacts of S-metolachlor (SMOC) and terbuthylazine (TBA) in the biochemical profile of *S. plana*. SMOC and TBA are active ingredients (a.i.) in several common herbicides, such as Primextra® Gold TZ, the most used in the farmlands that surround the Mondego estuary, in the coast of Portugal. The overuse of pesticides leads to contaminations in aquatic systems, affecting non-target species. *S. plana* was selected due to its key position in trophic webs, linking primary producers and consumers. Bioassays were performed exposing *S. plana* to a range of concentrations of each chemical for 96h. Fatty acids (FA) and carbohydrate composition were analysed in the survival organisms. Moreover, the analyses were performed in two types of tissues - the muscle and visceral mass of each organism. Muscle presents higher saturated fatty acids (C18:0, C20:0, C23:0 and C24:0) and unsaturated fatty acids (C22:1, C24:1n9, C22:2 and DHA) than visceral mass. Thus, muscle shows to be the most cost-effective tissue to detect the presence of SMOC and TBA in aquatic environments. Organisms exposed to SMOC present low highly unsaturated FA whereas organisms exposed to TBA showed low levels of monounsaturated FA

and polyunsaturated FA. In general, TBA exposure resulted in lower nutritious quality in terms of FA content and diversity than SMOC exposure. Similar changes have been reported in aquatic primary producers exposed to Primextra, thus, there is a risk of low occurrence of FA in aquatic environments exposed to this pollutant. Glucose is the only carbohydrate found in *S. plana*, expectable since glycogen is the main polysaccharide in animal tissues. Glucose content increase under chemical exposure. The sugar analyses also reported a decrease of glycogen. The present work presents tools to predict the presence of SMOC and TBA in aquatic systems and to go deeper at the knowledge of their biochemical impacts in non-target species and its propagation through aquatic trophic webs to the entire environment. FCT supported the study through the strategic projects UID/AMB/50017/2013, UID/MAR/04292/2013,UID/CTM/50011/2013 and POCI-01-0145-FEDER-007679, UID/QUI/00062/2013 granted to CESAM, MARE, CICECO-Aveiro Institute of Materials, and QOPNA, respectively. FCT funded A.F.C. Mesquita, A.M.M. Gonçalves and C. Nunes by doctoral (SFRH/BD/139831/2018) and post-doctoral (SFRH/BPD/97210/2013 and SFRH/BPD/100627/2014) grants, respectively.

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Copepod respiration at combined exposure to predation risk and copper

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Predation risk can potentiate copper (Cu) toxicity in copepod age at maturity. One suggested explanation is that predation risk synergistically interacts with anthropogenic stressors through increased metabolic demands and nutrient limitation. To identify the underlying processes of the potentiated Cu toxicity, we examined the combined effects of predation risk and Cu on copepod respiration. We used the harpacticoid copepod *Tigriopus brevicornis* as our model species. Adults individuals were incubated individually in OxoDish OD24 wellplates, and exposed for 15 hours to one of the treatments predation risk, Cu (20 $\mu g\ L^{-1}$), combined predation risk and Cu (20 $\mu g\ L^{-1}$), or control. Predation risk was simulated by exposing the copepods to water containing three-spined stickleback kairomones (i.e. chemical cues). We measured copepod respiration continuously while incubated using SDR SensorDish® Readers.. We also examined differences in respiration among males, females and pregnant females (i.e. females with egg sac). After terminating the experiment, individuals were photographed for subsequent length measurements. Preliminary results suggest that both predation risk and Cu increase respiration during single exposure, and effects are less than additive when combined. Nor are any respiration differences observed among males, females and pregnant females. We additionally examined if spawning rates or egg-emergence among females varied according to treatment, however, no patterns were evident from the results. Our results as presented here are preliminary and should be interpreted accordingly. While both stressors seemingly increase respiration, it does not seem to explain the potentiating effect of predation risk on Cu toxicity in copepod age at maturity.

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Response variability to multiple stressors exposure in wild fish: biological level and populations matter

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Aquatic organisms face multiple challenges in human-altered rivers such as abiotic (e.g. contamination) and biotic (e.g pathogens) stressors. Defense mechanisms against these stressors are likely to interact strongly but their interacting effects are still understudied, hindering our ability to predict the responses of wild populations to multiple stressors. Recent studies bring very variable results depending on the populations and biological level of organization considered. In this study we tested: i) how an experimental exposure to trace metals could affect the response (immunity, bioaccumulation, energy reserves, body condition, behavior) of wild fish across biological levels (molecular, cell, organ, individual) And, ii) how the past history of exposure to metal pollution would affect their plastic responses to the experimental contamination? We have predicted that the exposure to combined contamination and immune challenge would influence mostly labile traits such as behavior and physiology through shifts in energy allocation for investment in detoxification process and immune response. We also have predicted that populations of fish would display contrasted sensitivity to stressors. More specifically, fish originating from the most contaminated areas will be better able to cope with the experimental contamination than control unpolluted populations. Keywords : trace metal, immune challenge, sensitivity, stressors interaction

Advancing Chemical Substitution and Alternatives Assessment: Challenges, Opportunities, and Approaches

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Free-of charge online tools driving substitution

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With the development and global acceptance of the *SIN List* as a chemicals management standard, ChemSec has disclosed the pathway for progressive companies in order to future-proof the business. The SIN List has, since its launch in 2008, become the most important list of chemicals for companies to understand where to focus the efforts in chemicals management. With 15 000 users and the inclusion in many companies RSLs and MRSLs it has formed the basis for substitution work in many areas. In addition to this, the SIN List is an integral part in other sustainability efforts, such as eco labels, and has been endorsed by ECHA as a "driver for innovation". The most important contribution is probably the inclusion in Dow Jones Sustainability Index, where it forms the basis for the most important question in the chemicals sector. Undoubtedly, the SIN List has been a very important contribution in the work towards a reduced use of hazardous chemicals. Consequently, many companies work hard to substitute the hazardous chemicals in products and supply chains with safer alternatives. Until recently, alternatives have hard to find, even though numerous innovative companies develop sustainable solutions, offering products devoid of hazardous chemicals. The alternatives are often not known to a wider audience, due to lack of marketing opportunities and difficulties in bringing sustainable products to market. With our new tool, *ChemSec Marketplace*, we provide a solution to the contradiction between supply and demand for safer alternatives: a global B2B platform dedicated to realize the connection between solution providers and downstream users looking for solutions. Since the launch in the end of 2017, Marketplace has received a lot of attention. Several large chemical producers such as Chemours, Huntsman and Clariant use it to promote their safer alternatives. In addition to this, Marketplace has become the number one spot for innovative SMEs and start-ups to display their alternatives. The number of advertisements (as of Nov 2018) has reached well beyond 100 and the users are above 4 000 per month. The growth, both for users and ads, has been steady during 2018, and we foresee similar numbers for 2019.

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Integrating life cycle impacts into chemical substitution

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Increasing chemical and product diversity and complexity contribute to exposure of humans and ecosystems to chemicals in millions of product-chemical combinations worldwide. Exposure can occur via product use, or via environmental emissions along entire product life cycles. In response, the European Chemicals Agency outlined a "Strategy to promote substitution to safer chemicals through innovation". Chemical alternatives assessment (CAA) is a suitable tool for chemical substitution, but lacks indicators for impacts along chemical and product life cycles, leaving important tradeoffs unassessed. To address this challenge, exposure and life cycle impacts need to be consistently integrated into CAA. We systematically evaluate the scope of CAA and identify key elements for quantifying exposure and life cycle impacts. We use a tiered approach to assess for different scopes indicators, starting points and assessment outcomes. At each level, cumulative near- and far-field transfers and exposures are linked to chemical mass used in products, emissions, and toxicity information. Other impacts are screened for their contributions to product-related impacts per application type. We test our approach in a case study of plasticizer in vinyl flooring and outline future research needs to operationalizing a Life Cycle-based Alternatives Assessment (LCAA). We demonstrate that it is important to combine factors quantifying chemical mass in product, expsoure and hazard to allow for an overall screening-level ranking of alternatives based on using comparative metrics. Across 10 potential alternatives to DEHP, the non-phthalate plasticizers DEHA and 97A show hazard quotients up to a factor 30 lower. We show that plasticizers contribute between 55 and 85% to total human toxicity burden from flooring. Comparing toxicity with other life cycle impacts emphasizes the relevance of toxicity for the studied chemical-product application. Our results demonstrate how all relevant exposure pathways, populations, and life cycle impacts can be consistently considered to avoid burden shifting resulting from disregarding chemical and product life cycles, and to prioritize the most relevant life cycle impacts. As data and models are becoming more and more available, our appraoch constitutes a valuable starting point for quantitatively and consistently considering exposure and life cycle impacts in CAA. This can ultimately lead to a more comprehesive, yet rapid screening substitution assessment.

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Environmental impact assessment of substitution of hazardous substances in industry

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Following the pressure on companies for substitution of hazardous substances as demanded by the EU REACH regulation and other legal frameworks, there was a need for a methodology to assess the impacts of substitution to the environment. Research work was carried out within the project LIFE Fit for REACH, having an aim to encourage substitution of hazardous chemicals in small and medium enterprises (SMEs). The developed environmental impact assessment methodology was tested on a number of companies, including metal and food processing, production of construction materials and household chemicals. The companies involved had problems with carcinogenic, mutagenic, reprotoxic (CMR) substances, endocrine disruptors and volatile organic compounds (VOCs). It was proposed to integrate different levels of indicators, covering the causal chain between pressures (expressed as emissions of targets substances), environmental state (expressed as change in risk characterisation ratio and in environmental impact category indicator values), and response (expressed as amounts of substituted substances). In addition, occupational safety methodology considering the life cycle thinking, as adapted to EU, has been incorporated to cover the life cycle aspects of occupational safety due to accident risks. The methodology has been designed for the environmental impact assessment of substitution of hazardous chemicals. Overall, the indicators were useful in informing the effects of chemical substitution in different areas of impact by covering life cycle stages, major environmental impact categories and workers.The developed methodology can be adopted by various entities with relevant expertise who want to assess the wider environmental impacts of chemical substitution with a more complete set of indicators.

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The concept of essential use for determining when uses of PFASs can be phased out
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Per- and polyfluoroalkyl substances (PFASs) are a group of more than 4700 substances that have been produced since the 1950s and been used in a broad range of consumer products and industrial applications. Based on concerns regarding the high persistence of PFASs and the lack of knowledge on chemical structures, properties, uses, and toxicological profiles of the many PFASs in use, it has been argued in the Madrid Statement that the production and use of PFASs should be limited. It is, however, not practical to ban all uses of all PFASs in one step. If some uses of PFASs are found not to be essential to the functioning of today's society, they could be eliminated without having to first find functional alternatives. Elimination of non-essential uses of PFASs could form a starting point for a process that leads to a global phase-out. In this presentation, I will a) define the concept of essential use and b) apply the concept of essentiality to various use categories of PFASs to determine the feasibility of limiting use. Our approach for defining the concept of essential use is based on the example of the Montreal Protocol, where essential uses are defined. The two elements of an essential use under the Montreal Protocol are that a use is "necessary for health or safety or for the functioning of society" and that "there are no available technically and economically feasible alternatives". In this present work, to identify uses of PFASs that are non-essential, we combine the definition of essential uses with several categories of PFAS uses and ultimately derive 4 distinct use categories, which will be described in the presentation. For uses in categories 1-3, a phase-out of PFASs can be prepared because these uses are not necessary for the betterment of society, i.e., Health Safety, etc. or there are currently functional alternatives available that can be directly substituted into these products or applications. Category 4 are considered necessary and currently have no established alternatives to PFASs. However, this essentiality should not be considered as forever; rather, a constant pressure is needed to search for alternatives. In the presentation, I will provide a detailed description of several ongoing uses of PFASs and discuss whether the uses of PFASs are essential or non-essential based on classification according to the four use categories we have developed. We would like to expand this concept and apply it in detail to all use categories of PFASs.

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Stochastic High Throughput Stochastic Modeling of Human Exposure to Chemicals in Consumer Products
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Recently, increasing attention has been given to human exposure to chemicals in consumer products. Yet, Alternatives Assessment (AA), Risk assessment (RA) and Life Cycle Assessment (LCA) methods lack efficient and flexible approaches to quantify exposure and hazard for the many thousand product-chemical combinations that are currently in use. To address this gap, we present an operational matrix-based high-throughput framework efficiently coupling multi-pathway near-field (worker and consumer) with far-field (general population) exposures and with hazard data vald for thousands of chemcials. The developed assessment framework involving the following steps: a) We use the Stochastic Human Exposure and Dose Simulation Model (SHEDS-HT) to generate the number of users and the mean chemical mass used per user for a simulated US population of 25,000 persons for 9700 chemical-consumer product combinations. b) We calculate as element of a matrix the direct fractions transferred to other compartments and humans, using seven models (skin surface layer, object surface layer, article interior, indoor air, food contact material, pesticide residues, direct emission). Inverting this matrix yields Product Intake Fractions (PiF) for various receptors. c) We then determine the total intake dose per user and per average person in the population for the 9700 chemical-consumer product combinations from SHEDS-HT and d) We predict risks and damage (μDALY) per user and average population. Several of these chemical-product combination yield exposure doses that exceed the reference dose, thus leading to hazard quotient higher than one, the potential impacts on user ranging from less than 0.1 to more than 100 μDALY/pers/d. The average population dose accounting for both users and non-users\n intakes ranges from 8.2E-8 to 3.1 mg/kg_{BW}/d and is dominated by user contributions.The dose received by users and by average population can differ by several orders of magnitude. At population level, two chemicals lead to impacts of 120 to less than 13 μDALY/avg pers/d. These product-chemical combinations correspond to the highest impacts among the 9700 combinations and are those to further study and analyze in priority. Most contributing product usages are associated with personal care products, cleaning products, home maintenance and other home products. The developed approach proves a powerful tool able to analyze thousands of product-chemical combinations.

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Alternative assessment of hazard chemicals combining in silico tools with multicriteria decision analysis (MCDA): a case study of decabromodiphenyl ether (decaBDE) alternatives
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Alternative assessment is applied for minimising the risk of replacing a hazardous chemical with another problematic, hazardous chemical. High quality experimental hazard data, however, are usually unavailable, especially for newly produced or proposed alternatives. In such cases, obtaining *in silico* data is the only approach to fill in data gaps. An additional concern is how to reach a decision based on uncertain *in silico* data across multiple endpoints. To address these problems, this study explored the use of different multicriteria decision analysis (MCDA) methods within an alternative assessment framework. For this, sixteen alternatives to a widely used flame retardant - decabromodiphenyl ether (decaBDE) - were considered as the case chemicals. This study also discussed what endpoints should be included for assessing the hazard. The hazard endpoints include not only persistence (P), bioaccumulation potential (B) and toxicity (T) which are generally considered by existing alternative frameworks, but also the mobility in water (M) which is fairly recent, and to our knowledge this is the first study to include M within an alternative assessment. Experimental data were collected from free-access databases and *in silico* data were calculated by open-source software or platforms. As expected, the data distribution of experimental data was uneven and large data gaps existed. *In silico* data were calculated for five P criteria, one B criterion, 13 T criteria and one M criterion and were able to address these data concerns. Three MCDA strategies were tested in this case study: heat mapping, MAUT and ELECTRE III. The heat map indicated that none of the target chemical is perfectly sustainable. MAUT and ELECTRE III indicated that the case chemicals include potential regrettable substitutes but also some potential less hazardous alternatives. In aggregate the results of these three MCDA approaches provided meaningful insights in how to use *in silico* data to make an appropriate final-decision strategy for a selection of alternatives.

New Insights into Chemical Exposures over Multiple Spatial and Temporal Scales

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Spatial variability of ecosystem exposure to home and personal care chemicals in Asia
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There are currently limitations in spatial and temporal resolution of environmental exposure models due to significant variabilities and model uncertainties. Here we present the updated Pangea multi-scale multimedia model based on the more spatially resolved, catchment-based hydrological HydroBASINS dataset covering the entire globe. We apply it to predict spatially-explicit exposure concentrations of two home and personal care chemicals in rivers across Asia: linear alkylbenzene sulphonate (LAS) and triclosan (TCS), and test its potential for identifying/prioritizing catchments with relatively high exposure concentrations. In addition, we also identify the key parameters in the model framework driving predicted high concentrations and perform Monte Carlo uncertainty analyses on emissions and other non-spatial model inputs. The updated combination of Pangea with the HydroBASINS hydrological data represents a substantial improvement for modelling substance fate, with higher resolution and improved coverage in regions with lower flows, with good agreement with monitored concentrations for TCS in freshwater (r=0.74) and sediment (r=0.9). The ranking of water basins by predicted PECs showed highest concentrations (Indus, Huang He, Cauvery, Huai He and Ganges), one to two orders of magnitude greater than the water basins with lowest predicted PECs (Mekong and Brahmaputra). Emissions per unit volume of each catchment, chemical persistence, and river discharge were deemed to be the most influential factors on the variation of predicted PECs. For rapidly degraded LAS, only a few upstream catchments situated in proximity contribute substantially to the predicted PECs, whereas a much larger number of upstream catchments over 2000 km contribute to the predicted PECs for more persistent TCS. Focusing on the Huang He (Yellow River) water basin, uncertainty confidence intervals are much lower than the variability of PECs across the Huang He catchments.

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The first global study of pharmaceutical contamination in riverine environments
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Significant knowledge gaps exist regarding the occurrence of APIs in the environment, particularly in low to middle-income countries and those outside of N. America and W. Europe. Of the 196 countries recognised by the United Nations, data for the occurrence of pharmaceuticals is only available for 71. Here we present the first global pharmaceutical monitoring study in order to identify hot-spots of pharmaceutical contamination in rivers worldwide. Involving a consortium of 62 collaborators, the study composed of 71 sampling campaigns performed across 61 countries covering all continents (n=537). Thermally-insulated water sampling kits were sent to partner intuitions who then collected 10mL of water from 5-10 locations along their study river(s). All samples were filtered in-field using a glass microfiber syringe filter (0.7μm pore size). Samples were sent frozen and on ice to the University of York (York, UK) for quantification of 61 APIs using an in-house validated direct-inject HPLC-MS/MS method. At least one API was detected in all of the countries analysed and as many as 33 were detected in a single catchment (Lahore, Pakistan). Highest sum concentrations were found in rivers suspected to receive discharge from pharmaceutical manufacturing facilities in Lahore in Pakistan, Delhi in India and Lagos in Nigeria (Figure. 2). The most frequently detected API in Africa was the antibiotic ciprofloxacin with a detection frequency (DF) of 55%, in Asia the anti-hyperglycaemia drug metformin was most frequently detected (DF=79%). For the other continents, carbamazepine (DF=78%), gabapentin (DF=77%), paracetamol (DF=54%) and ciprofloxacin and fluconazole (DF=67% each) were the most frequently detected APIs in Europe, Oceania, N. America and S. America respectively. Initial analyses indicate that per capita gross domestic product on a purchase power parity-basis may reflect API contamination where low 'middle-income' countries show highest sum API concentrations. This may be reflective of the fact that while these countries have ready access to medicine, the level of investment in and regulation of wastewater treatment remains low. To the best of our knowledge, this is the first study exploring API contamination of rivers on a global-scale. Our results clearly show that the occurrence of pharmaceuticals in rivers is an issue that affects the whole planet and much work is needed to identify potential impacts in poorly-studied regions.

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Pharmaceutical translocation from wastewater into bee hives
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The potential for pharmaceuticals to accumulate in pollen and nectar has been a topic that has been very much over looked with research predominantly focussing the accumulation of these chemicals in shoots and roots. Nevertheless, a similar chemical profile and known ability to cross the root membrane and translocate within a plant would suggest that like insecticides, pharmaceuticals, have the potential to accumulate in nectar and pollen. There is therefore an urgent need to establish the potential for these biologically active chemicals to accumulate in nectar and pollen and to understand the subsequent exposure to bees and the beehive. Residues of carbamazepine were detected in nectar (1.75 – 371.41 ng/mL) and pollen (0.02 – 30.85 μg/g) sampled from zucchini flowers (*Curcubita pepo*) exposed to carbamazepine spiked soil (0.5 – 20 mg/kg). It was estimated that the concentration of carbamazepine in nectar and pollen would be 70 fg/mL and 880 fg/g respectively, under a realistic soil exposure of carbamazepine (20ng/kg) based on a linear relationship between carbamazepine concentrations in soil and nectar/pollen (R^2 >0.99). Incorporation of these estimated carbamazepine residues into the BEEHAVE modelling framework enabled the simulation of the potential transfer of carbamazepine to 11 beehives at a landscape scale over three simulated years (2010, 2011, 2015). The amount of carbamazepine gathered increased exponentially during the flowering period of zucchini reaching a maximum carbamazepine concentration in a single hive of 86.97 ng. Annual average concentrations ranged between 8.73 and 30.65 ng across the 11 hives in the 5km landscape. Carbamazepine accumulation varied between hives within the same year by up to two orders of magnitude. These findings demonstrate the importance of the hive location, weather, and time on carbamazepine accumulation. Carbamazepine residues in the pollen contributed to on average 5-6 times more carbamazepine in each colony than nectar on an annual basis. This work describes a fundamental first step in the quantification of pharmaceuticals in nectar and pollen and demonstrates that more work is urgently needed on this topic. A detailed understanding of the potential transfer of pharmaceutical residues to the beehive will enable scientists and regulators to be able to design experiments to consider the risk of this exposure (e.g. larval toxicity) by considering realistic exposure doses of pharmaceuticals.

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Understanding Insect-Mediated Contaminant Flux: When, Where, and How to Look for Contaminant Export by Emergent Aquatic Insects
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The exchange of subsidies across the land-water interface is a key driver of community dynamics and ecosystem processes in both aquatic and riparian habitats as organic matter, nutrients, and animals flow between these systems. However, biological vectors can also transport contaminants across the land-water boundary. A classic example of contaminant-linked subsides came from early studies of egg shell thinning in birds. In these studies, reproductive failure in piscivorous birds (e.g., eagles and pelicans) was linked to DDT exposure from consumption of fish that had accumulated in the aquatic ecosystem. Like birds, adult aquatic insects have been shown to transport significant quantities of contaminants such as PCBs out of aquatic environments, thereby linking aquatic contamination to terrestrial predators via a phenomenon called insect-mediated contaminant flux. Here, we present: 1) a conceptual model for insect-mediated contaminant flux based on four critical ecological processes that govern export of contaminants. 2) A decision-making tool to assess the likelihood of terrestrial risk via insect-mediated flux. This tool, named the Riparian Impact Test, integrates the technical science of the conceptual model into a step-wise series of four questions to aid risk assessors. 3) Practical considerations for risk assessors, including how to best sample for insect-mediated contaminant flux at a specific site.

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Towards a temporally and spatially resolved Nested Exposure Model for organic contaminants in Arctic marine ecosystems
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There is concern over possible effects on Arctic ecosystems from exposure to persistent organic pollutants (POPs) and chemicals with similar properties. To enable scientifically sound response strategies, a mechanistic understanding of the complete link between chemical emissions and resultant concentrations in ecosystems is fundamental. The main objective of this study is to develop, evaluate, and apply a new Nested Exposure Model (NEM) consisting of (i) a global spatially and temporally resolved module for fate in the physical environment and (ii) a bioaccumulation module, currently developed for European Arctic marine ecosystems. Here, evaluation of the bioaccumulation module across trophic levels and time is presented. The dynamic model ACC-HUMAN Arctic was adapted to represent ecosystems in the Svalbard and Barents Sea areas. The model includes herbivorous, omnivorous, and carnivorous zooplankton, fish (herring, capelin, polar cod, and Atlantic cod), and ringed seal. Model performance was evaluated for selected polychlorinated biphenyls (PCBs) in Kongsfjorden (79 °N 11 °E) and in the wider Barents Sea. As suitable long-term empirical time trends for PCB concentrations in the physical environment remain fragmented, different scenarios were constructed to evaluate the model. The best model performance was achieved when running the model using as input the time-trends of PCBs in seawater predicted for the Baltic Sea region, but adjusted to concentrations observed in Arctic seawater. Using this scenario, the bioaccumulation module performed well in reproducing bioaccumulation of PCBs in the Kongsfjorden food web. The ratio of predicted to measured concentrations ranged from 0.3 – 6.7 except for PCB 28 in ringed seal (ratio of 16-19). Predicted

time-trends of ΣPCB_6 and ΣPCB_7 were also in good agreement with measurements from the Barents Sea for all fish species. The good model performance across trophic levels and time is promising for further expansion of the model to include (i) variation in space through connection with the physical module of NEM, and (ii) Arctic marine top-predators, the terrestrial environment, and human exposure. The physical module of NEM is still under development, but preliminary results will be presented. When finalized, we hope that the NEM model will be a useful tool for scientific and regulatory communities interested in understanding and protecting ecosystem and human health from legacy and emerging organic contaminants.

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What can we learn from the temporal trend of human exposure and concentrations of PBDEs?
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A number biomonitoring programs have observed declining trends in concentrations of polybrominated diphenyl ethers (PBDEs) in human tissues in response to national regulation or restrictions; however, the rate of decline often varies between congeners, ages, and countries. In this presentation, we systematically investigate the mechanisms responsible for such variability through modeling time-variant lifetime exposure to four PBDE congeners (47, 99, 153, and 209) via multiple exposure pathways (e.g., “far-field” food consumption and “near-field” non-dietary object-hand-mouth contact) from multiple lifecycle stages (industrial processes, the indoor and outdoor use phases, and waste disposal) in interconnected indoor, urban and rural environments. Our model simulation indicates that, after the ban of new PBDE uses, concentrations of congeners with (i) a dominant exposure contribution from far-field exposure routes and (ii) higher resistance to biotransformation in the human body, tend to decline slower. Because of continued release of PBDEs from disposed waste after the end of the lifespan of consumer articles, contamination in far-field environmental media (e.g., rural air and freshwater) and organisms (i.e., the source of foodstuffs) outlasts that in near-field environmental media (e.g., indoor air and surfaces). We, therefore, can expect a chemical with dominant exposure through far-field routes to exhibit a slower rate of decline. This can be the case for (i) more persistent and less volatile congeners (e.g., BDE-153, which is more bioaccumulative and with lighter emissions at the indoor use phase than other congeners), (ii) exposure of adults (due to higher food consumption rate and less frequent object-hand-mouth contact), and (iii) regions with considerable far-field end-of-life emissions. The presentation illustrates how exposures over multiple spatial and temporal scales interact to determine the dynamics of the human body burden of PBDEs.

Revision of the EFSA Guidance Document on Birds and Mammals

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Revision of the EFSA guidance document on 'Risk Assessment for Birds and Mammals'
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In order to gain EU Level approval of an active substance under Regulation (EC) 1107/2009, it is necessary to demonstrate that there are no unacceptable risks to bird and wild mammals. After nearly ten years since the publication of the EFSA guidance document titled ‘Risk Assessment for Birds and Mammals’. (EFSA, 2009). EFSA has received a mandate to update it. The main aim is to revise the guidance with the latest scientific knowledge and to provide further clarifications on aspects of the guidance which were commonly misinterpreted. A fundamental step in the revision is the consultation of stakeholders to ensure that the revised guidance is fit for purpose. To this end, EFSA launched a public consultation of the EFSA (2009) guidance document in 2017. Another public consultation is planned on the draft revised guidance. A summary will be given of the plan for the revision of the guidance document and the key issues identified by the public consultation which require consideration during the development of the revised guidance.

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Revision of the EFSA Guidance Document on Birds and Mammals: Industry view
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The EFSA Guidance Document on the Risk Assessment for Birds and Mammals [EFSA, 2009)] (EFSA GD) has been used for more than 8 years in the EU for the evaluation of active substances and Plant Protections Products. This GD is now under formal revision by EFSA. SETAC Europe 2019 is an appropriate forum to share among risk assessors and other stakeholders the experiences with the use of the current GD and provide recommendations and proposals for its ongoing revision. Industry will contribute by sharing the experience from risk assessors who have been using this guidance on daily basis, highlighting those areas where the revision should put more emphasis. Our experience is mainly based on the scientific assessments conducted by risk assessors from regulatory bodies in the EU (Member States and EFSA) as presented in regulatory documents for the registration of active substances and PPPs. The basic principles of the risk assessment in the current GD (conservative tier 1 calculations for the in-crop acute and reproductive risks and options for higher tier refinement) have in general worked adequately and should be maintained. The major issues have occurred with the refined risk assessments, particularly for the reproductive (long-term or chronic) risk. The ongoing revision of this GD should take into consideration the weaknesses observed during its implementation by risk assessors during the last years: different interpretation and use of refinement options by different risk assessors from authorities with the same data. To address these issues, the revised GD should provide a more detailed technical description and guidance on some refinement parameters and better define which options for refined risk assessment could be widely used and accepted by risk assessors. The main refinement parameters that, in the opinion of risk assessors from industry, require particular attention for the revision are: selection of focal species of birds and mammals, relevance of the vole scenario, ecotoxicologically relevant reproductive endpoint for mammals, residues in food items, proportion of food (PT) taken in treated areas, field effects studies and modelling.

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Collection of ecological data and residue data in food items to be used in pesticide risk assessment for birds and mammals
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The current EFSA Guidance Document (GD) for the risk assessment of plant protection products for birds and mammals (EFSA, 2009) follows a tiered approach. The GD provides options for higher tier risk assessment, in cases where a low risk cannot be identified with the first tier risk assessment. The refinement steps may include: (1) the identification of specific focal species (FS), (2) the use of measured residues and measured residue decline in FS food items, (3) field information on the composition of diet obtained from an area treated with pesticide (PD factor), and (4) field information on the proportion of an animal’s daily diet obtained in a real habitat treated with pesticide (PT factor). In order to enable proper and consistent use of such data, EFSA initiated a project that was conducted from 2016 to 2018. The screening of data submitted for approval/authorization of pesticides yielded close to 1,000 relevant regulatory papers (i.e., mostly confidential reports by companies) and about 600 relevant public papers cited in the Draft Assessment Reports (DAR’s) or Renewal Assessment Reports (RAR’s). The searches in the public literature bibliographies yielded almost 30,000 ‘hits’ but only 1-2% of these papers were relevant enough to be screened and summarised. The final MS-Excel databases with relevant data for birds, mammals and residues contain 3196, 1439 and 2937 records respectively. The residue database contains entries on 190 active substances and metabolites. Fungicides, herbicides and insecticides represent 99% of all records. For crops, the highest number of records in the databases stem from residue studies on cereals, vegetables, maize, in orchards and vineyards, and for birds and mammals, from grassland studies. The ecological databases contain data on about 150 bird species and 70 mammal species. The food items represented in the ecological databases included all major food item categories, but especially crop seeds and plants, both monocotyls and dicotyls. This may be due to the fact that many studies were conducted for granivorous and herbivorous animals. The focus of the records in the residue database was on monocotyls and dicotyls and on fruits, with only few records on arthropods and very few on weed/crop seeds and worms. The data collection could serve as a valuable basis for future developments in the risk assessment for birds and mammals.

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Industry data for the revision of the EFSA GD on Birds and Mammals
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The EU risk assessment guidance for birds and wild mammals (EFSA 2009) will be revised, and therefore the present guidance should be checked whether the current parameters and recommendations should be updated. For this revision, industry is providing data via two different processes. First, individual companies have provided more than 800 study reports to the EFSA tender for data compilation. Second, the ECPA companies are jointly compiling, analysing data and making publications; data, calculations tools and reports are offered to EFSA for the revision of the GD. Four of these projects are presented here: 1) Residue dissipation (DT50): The EFSA GD assumes a "generic" DT50 of 10 days for dissipation of residues from food items (eg foliage). The underlying residue data were largely from older studies with unclear relevance for modern agriculture in Europe. We evaluated more modern and relevant foliage residue decline studies and found that the generic DT50 of 10 days is indeed a conservative assumption. No significant differences were found for the DT50 in monocot or dicot foliage, or between the Northern or Southern EU residue zones. 2) Residue estimation for fruits: The current EFSA GD provides RUDs for residue estimation on bird and mammal food items, including fruits of different types. The ECPA team has evaluated residue studies with fruits like grapes, 'other berries', gourds, and large fruits from orchards. The large dataset (n≥100 trials per food item) revealed significantly lower mean RUDs compared to the current default values, particularly for gourds and large fruit from orchards. 3) LD50 extrapolation for mammals: The current EFSA GD provides extrapolation factors for the acute oral toxicity tests in birds to determine a point estimate for the LD50 for studies in which no or little toxicity was observed. The ECPA expert team has now established such extrapolation factors also for mammals. 4) 21-d PT for long-term risk assessment: The "PT-factor" in the current EFSA GD represents the portion of time an animal spends potentially foraging in a treated field. Individuals tracked over more than a single day often show significant variation from one day to another. Therefore the ECPA team has developed an evaluation method that accounts for both the inter-individual and the intra-individual variation. Here we present follow-up work including also other species of potential concern (eg, skylarks or hare).

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From focal species to effects studies: important points for the revision of the EFSA Guidance Document on B&M

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The planned update of the Guidance Document (GD) on the risk assessment (RA) for birds and mammals for plant protection products (PPPs) is a great opportunity to clarify topics which are not specified in detail in the current version. For example, field effects studies are one acknowledged option to verify acceptable risk, but no detailed information is given about their required test design. Therefore their acceptance by member states varies due to doubts regarding the study validity. However, field effects studies can measure potential effects of PPPs under realistic field conditions and reduce the use of over-conservative assumptions, so they are a valuable tool to cover uncertainties. Major critical points of a field effects study are the selection of the focal species and the adequate study design and sample size. The focal species should represent the worst-case in terms of exposure and by doing so, it becomes protective of other species. However, this concept is not always applied by the regulatory authorities, which shows the need to strengthen the standards given in the GD. On the other hand, the study design needs to be defined according to specific concerns in each case. The minimum number of necessary individuals or sites can be estimated by means of statistical simulations, based on industry data or literature information. This is an important factor, as having the right sample size allows to cope with the high variability of natural conditions. **Acute effects on birds or mammals** can be studied by using the technique of radio tracking. Any lethal but also sublethal effects are detected by regular checks of individual activity. **Reproductive effects on small mammals** can be tested using capture-mark-recapture technique and either an intensive approach or a landscape approach (different in the number of sites and in the trapping scheme) depending on the focal species and the target crop. **Reproductive effects on wild birds** can be tested by nest monitoring studies. The reproductive performance following a PPP application can be shown using the same endpoints as in tier1 avian reproduction tests and for further key parameters like nest survival and number of fledglings per pair. Field effects studies provide an accurate assessment of the impacts of PPPs in the nature. They add realism in the otherwise theoretical risk assessment and therefore further guidance should be given in the context of the revision of the EFSA GD on birds and mammals.

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How to use radio tracking data from multiple tracking sessions in refining the risk to birds and mammals from the use of pesticides

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One of the refinements used in the bird and mammal risk assessment for plant protection products is to consider the amount of food that an individual obtains from a treated field. This is usually referred to as PT or the proportion of an animal's daily diet that is obtained in habitat treated with pesticide. It is usual for this refinement to be based on radio-tracking of individuals of a species of concern or relevance. The radio-tracking data indicate a "day in the life" of an individual, however the data are often used to refine the long-term/reproductive risk assessment, hence there is a potential mismatch between the time period used in the refinement and the risk assessment. This presentation will describe how radio-tracking data have typically been used by regulatory authorities in bird and mammal risk assessment. Recently Ludwigs *et al.* (2017) have published work regarding how to produce a 21-day time averaged value for PT using a Monte Carlo method, which may be relevant for the long-term/reproductive risk assessment. This method uses data from multiple tracking sessions for individual birds/mammals. The European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals (2009) does not specify how to use radio-tracking data from multiple sessions for the same individual, which can lead to inconsistencies in interpretation. The UK Health and Safety Executive (HSE) has therefore reviewed the method described by Ludwigs *et al.* in a position paper. While this method raises important issues and illustrates how more use can be made of PT datasets, there are several concerns regarding how the method has been implemented. This presentation will discuss the method proposed by Ludwigs *et al.* and the concerns identified with this approach by UK HSE. These concerns have been broadly grouped into toxicological, ecological and statistical points. The Ludwigs *et al.* paper represents an important development in the use of radio telemetry data but does not detail a universal method that can be applied to any PT dataset. The points raised in this presentation should be further considered when designing a radio-tracking study or when using the data from such a study in risk assessment.

Epigenetic and Evolutionary Effects of Environmental Stressors on Environmental and Human Health

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Genomic signatures of heavy metal tolerance in the soil arthropod Orchesella cincta

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A major component of urbanization encompasses the accumulation of toxicant substances. Heavy metals have received considerable attention as they are known to accumulate at ever increasing rates in the environment. Exposure to these toxicants constitute novel environments and the degree to which organisms may evolve genetic tolerance towards these stressors remains poorly understood. Exposed populations of the soil arthropod *Orchesella cincta* evolved metal tolerance through increased metal excretion efficiency and, consequently, a decreased cadmium (Cd) growth reduction. Previous research further demonstrated that the observed tolerance of adapted populations largely involves constitutive over expression of genes involved in metal detoxification. Yet, the degree to which this tolerance is reflected at the genomic level remains at present unknown. Therefore, we present the analysis of genome wide allele frequency data from three adapted and four reference populations, obtained by Restriction-site Associated DNA sequencing (RADseq), to test for genetic signatures of selection. Mapping SNP loci that confer signatures of genetic adaptation to the fully annotated genome of this species allows association between the genomic targets of selection and the genes that show altered constitutive and induced expression in response to metal exposure. Interestingly, several of these genes were already identified in a previous transcriptome analysis to be among altered gene transcripts in metal tolerant phenotypes. These results do not only inform us about the capacity of natural populations to genetically adapt to human stressors, but also how integration of different approaches (phenotypic/outlier locus detection/transcriptomics) may reveal signatures of adaptation.

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Multigenerational exposure to CuO NMs with Enchytraeus crypticus - nanomaterials and epigenetics?

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Chemical stressors can induce epigenetic changes, i.e., genomic changes that are transferred to the next generation, even when the stressor is removed: transgenerational. This is well-known for humans and carries additional concern, but has been especially less studied in environmental relevant species. In this study we assessed the epigenetic potential of Cu materials in terms of global and specific methylation, combined with gene expression allowing to target genes involved in among others DNA methylation, histone modifications and non-coding RNA. We exposed the standard soil model species *Enchytraeus crypticus* in a multigenerational (MG) test to Cu (copper oxide nanomaterials (CuO NMs) and copper salt ($CuCl_2$)). Sub-lethal effect concentrations (start generatoion) were used for 4 consecutive generations (plus 2 generations transferred to clean media, F1 to F7 generations in total). The results showed that MG exposure of Cu caused an increased in the average global DNA methylation but significance not shown. Gene expression showed changes in the epigenetic, stress and detoxification gene targets, depending on the generation and Cu form, also occurring in post-exposure generations - transgenerational. Differences observed between Cu forms indicate nanoparticulate specific effects. Again, highllighting the importance of long-term studies for risk assessment.

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Exposure to copper during embryogenesis caused a transient increase in tolerance in the three-spined stickleback (Gasterosteus aculeatus) for two subsequent generations

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Exposure of wildlife to environmental pollutants can occur continuously or intermittently over long periods of time, and their surivival is dependent on their ability to adapt to these exposures. We conducted a series of copper exposures in stickleback (*Gasterosteus aculeatus*) to investigate whether prior exposure can result in altered susceptibility later in life and/or in subsequent generations. A copper pre-exposed population was generated by exposing stickleback embryos to low-level copper during early life (0-9 days post-fertilisation), causing ~1.2% mortality, ensuring that selection for a tolerant genotype did not occur. These F0 fish were then kept under control conditions until maturity (9 months) and bred to produce F1 and F2 adults with no further exposure. A control population was generated in parallel. To assess altered susceptibility within a generation, F0 adults from the pre-exposed and control populations were re-exposed to copper. Prior to re-exposure, basal tissue copper content was significantly higher in pre-exposed fish compared to controls. In addition, pre-exposure affected copper content upon re-exposure, suggesting that embryonic exposure altered copper metabolism later in life. We hypothesise that this change is due to an altered physiological response, potentially caused by stable epigenetic alterations induced during early life exposure. This is currently being investigated using RNA-seq and whole genome bisulfite sequencing. To assess altered susceptibility of future generations, we exposed embryos from both the pre-exposed and control populations to copper during early life. F1 embryos from pre-exposed parents were significantly more tolerant to copper compared to controls. This tolerance was maintained in the F2 generation, but not the F3 generation. We hypothesise that the multigenerational tolerance observed up to the F2 generation was caused by parental effects, owing to altered copper handling and increased copper tissue burden in F0 adults, resulting in secondary exposure of F1 embryos and their germ cells which gave rise to the F2 generation. Our data support the hypothesis that exposure to low levels of copper during early life has the potential to reduce the susceptibility of vertebrates to subsequent exposure across generations, potentially via a combination of epigenetic, physiological, and maternal effects.

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Bioaccumulation and morphological traits in a multi-generation test with two Daphnia species exposed to Lead

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Anthropogenic pressures negatively affect the environment in different ways and at different levels. Lead (Pb) is a non-essential toxic metal being often detected in aquatic ecosystems. Xenobiotics like Pb can induce deleterious effects in different ways to different species, include close phylogenetically related species. In this study, two daphnid species, the temperate *Daphnia magna* and the tropical *Daphnia similis,* were used to assess a long-term Pb exposure, within a nine generations exposure to 50 μg/L. Besides Pb body burdens, effects were evaluated looking at non-commonly used endpoints like granules in the dorsal region of neonates, reddish extremities, carapace deformation and morphology, production of males and ephippia (or dormant haploid egg), changes in the eggs' color and abortion. The test was conducted under a usual and restricted food regime ($3x10^5$ and $1.5x10^5$ cells/mL). After 5 exposure generations, neonates were changed to a clean media for three other generations (F6 to F9) to assess recuperation. Negative and adverse effects occurred, but no disparity between species was shown. The *D. magna* Pb accumulation showed different patterns regarding food regime, being faster under usual food, and reaching a saturation point, whereas a gradual increase occurred under food restriction. A full retrieval occurred regarding Pb in *D. magna*, since no difference between control and recovering organisms was evidenced (Pb body burdens). Long-term Pb exposure led to carapace malformations, Pb aggregation in neonates' dorsal region, reddish extremities, production of males, ephippia (or dormant haploid egg), aborted eggs, and changes in eggs' color (green and white). Food restriction also induced the production of males. Reddish extremities disappeared in recovering organisms and ephippia (or dormant haploid egg) did not occurred during the recovery period. Bioaccumulation of Pb and adverse effects occurred at 50μg/L, although safety threshold reported for instance in the Brazilian legislation are within this Pb level, and scientific literature report occurring above 400 mg/L of Pb.

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Intra-generational effects of early life-stage exposure to tebuconazole on reproductive capacity of Zebrafish (Danio rerio)

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Tebuconazole (TEB) is fungicide used globally in agriculture to control for pathogenic species common to a variety of fruits, vegetables and cereals. It elicits endocrine disrupting effects through inhibition of the activity of cytochrome P450 enzyme, aromatase, which converts testosterone into 17β-estradiol (E2). Research suggests that exposure to endocrine disrupting compounds during early stages of development can impair reproductive physiology of adults. One mechanism of this effect is alterations to the DNA methylome that result in permanent reprogramming of gene expression. The goal of this study was to determine whether exposure to TEB during early stages of development affects the reproductive capacity of sexually mature adult Zebrafish (*Danio rerio*), and to investigate the mechanistic basis of any effects. Embryos were exposed to either a control (0 μg/L), 10 μg/L, or 1000 μg/L of TEB from 1-hour post-fertilization until 24-hours post-hatch. Larvae were then transferred to uncontaminated water and reared until sexual maturity. In embryos and adults, there were no significant effects of either concentration of TEB on mRNA abundance of genes along the HPGL axis or E2 concentration in plasma of adult fish. The mRNA abundance of ten-eleven translocase (*tet*) *3* was significantly greater in embryos exposed to 10 μg/L, and mRNA abundance of DNA methyltransferase (*dnmt*) *3b1*, *tet1*, *tet 2*, and *tet 3* were significantly greater in embryos exposed to 1000 μg/L. In adults, mRNA abundance of *tet 1* was significantly reduced in gonads from males exposed as embryos to 1000 μg/L. In brain of adults exposed as embryos to 10 ug/L, there was a significant decrease in mRNA abundance of *dnmt 2* and *dnmt 3b4* inmales and a significant increase in mRNA abundance of *dnmt 3a2* and *dnmt 3b1* in females. In brain of adults exposed as embryos to 10000 ug/L, there was a significant decrease in *dnmt 2, dnmt 3b4, tet 1*, and *tet 2* in males, and asignificant decrease in mRNA abundance of *dnmt 3a1, dnmt 3b2, dnmt 3b4, tet 1*, and *tet 2* in females. Using reduced-representation bisulfite sequencing (RRBS), 16 differentially methylated regions (12 hypomethylated and 4 hypermethylated) of the genome were identified in gonads of adult females exposed as embryos to 10 ug/L of TEB. Overall, results of this study suggest that the DNA methylome is a target of TEB during early stages of development but effects on DNA methylation do not appear to cause impaired reproductive capactity.

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DNA- and RNA-methylation in Baltic amphipods by using liquid chromatography coupled to high resolution mass spectrometry

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Adductomics can be defined as the screening of all additions (adducts) on a biomacromolecule. We have developed a liquid chromatograph-high resolution mass spectrometry (LC-HRMS) method for DNA- and RNA-adductomics using an Orbitrap Q Exactive HF mass spectrometer equipped with a heated electrospray ionization source. In this method we screened for adducts by taking advantage of the common deoxyribose (116.0473 Da) or ribose (132.0423 Da) moiety present in DNA- or RNA-nucleosides, respectively, after enzymatic hydrolysis of the biomacromolecules and chromatographic separation. The developed method was able to resolve DNA- and RNA- epigenetic markers, namely 5-methyl-2'-deoxycytidine (5-me-dC), N^6-methyl-2'-deoxyadenosine (N^6-me-dA), 5-methylcytidine (5-me-C) and N^6-methyladenosine (N^6-me-A), with high mass accuracy (< 3ppm). Subsequently, the developed method was applied to amphipods *Monoporeia affinis* collected within the Swedish National Marine Monitoring program having aberration frequency ≤5%. Twenty one females with the embryo brood removed and their broods were analyzed separately for DNA methylations (5-me-dC and N^6-me-dA) and RNA methylations (5-me-C and N^6-me-A). The extraction method employed with Chelex resin allowed for obtaining

sufficient amount of DNA and RNA from the same female or brood. Mean percentage (± standard deviation) of methylation corresponding to the unmethylated nucleoside [in the form of 5-me-dC×100/(dC+5-me-dC)] was approximately 11 (±3)% 5-me-dC, 0.023 (±0.013)% N^6-me-dA, 1.3 (±0.2)% 5-me-C and 0.94 (±0.17)% N^6-me-A in the females, and 6.7 (±2.2%)% 5-me-dC, N^6-me-dA below quantification limit, 17 (±12)% 5-me-C and 1.8 (±1.2)% N^6-me-A in the embryo broods. The relatively high standard deviation in the embryo broods is attributed to differences in their developmental stage and possible matrix effects. Nevertheless, the results can be used to compare the ratio of 5-me-dC to 5-me-C and 5-me-dA to 5-me-A in the females and 5-me-dC to 5-me-C in the broods. Whether the DNA- to RNA-methylation ratio can be used as a biomarker, e.g., for reproductive toxicity in the Baltic amphipods needs to be investigated.

The Environment as a Reactor Determining Fate and Toxicity of Nanomaterials (II)

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Determining the nanoparticle-algae interaction in the bioaccumulation process

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The use of engineered nanomaterials (NMs) raises concern regarding their fate in environmental compartments and their potential effects on aquatic organisms. One of the singularities of these substances is their duality of action, both physical and chemical. In the chain of events leading to the occurrence of a deleterious effect, the interaction between a NM and a biological organism is one essential event. Therefore, understanding the links between the properties of NMs and the physical interaction phenomenon (adhesion, internalization) with aquatic organisms, is essential in a context of *a priori* hazard assessment and for the categorization of these substances within a regulatory purpose. This work aims at determining the interaction of various NMs with freshwater microalgae, to gain a better understanding of its implication in bioaccumulation and biological effect. Green algae with different cell wall composition were separately exposed to different NMs suspensions. Flow cytometry and microscopic observation showed evidence of NMs-algae interaction. A dose-dependent response is observed for every type of NMs and a cell wall specificity is showed. So far, two parameters were identified as having a major role in the NM-algae interaction: i) the cell wall composition, as each algae had a different response to the set of tested NMs; and ii) the NMs physic-chemical properties; because for a given algae cell wall type, a different degree of increased algal complexity was observed according to each NM.

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The role of invertebrates in a tiered bioaccumulation testing strategy for engineered nanomaterials

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Due to the increasing usage of manufactured nanomaterials (MNMs) there is an mandatory need for testing the bioaccumulation potential of this materials within the regulatory frame of REACH. The bioaccumulation potential is usually expressed as bioconcentration factor (BCF) determined in flow-through studies with fish according to OECD 305 [1]. The increasing number of different MNMs would lead to an unrealistic workload for testings. Handy et al. 2018 [2] proposed a tiered aproach including stategies for the reduction of animals for in vivo testing. As part of this study, bioaccumulation tests were performed with a range of invertebrate organisms to elucidate the bioaccumulation potential of different MNMs. Based on the results obtained, bioaccumulation endpoints such as bioaccumulation factors (BAF) or biomagnification factors (BMF) are calculated and compared. The pros and cons of the different test systems are discussed. Bioaccumulation studies were carried out with silver MNMs (NM300K) and titanium dioxide MNMs (NM105). The test media containing the particles were applied in studies on the water flea *Daphnia magna*, the freshwater amphipod *Hyalella azteca* and the freshwater bivalve *Corbicula fluminea*. Both compounds showed different elimination behaviour which provide clear indications regarding the potential bioavailability of the test compounds. Bioaccumulation endpoints could be calculated, however, the presence of MNMs remaining in the gastrointestinal tract my confound the total burden measurement. Bioaccumulation studies with invertebrate species can be carried out as part of a tiered bioaccumulation testing strategy. However, test systems have differing suitability for testing MNMs which may require specific adjustments of the test systems or the use of additional analytical approaches to guarantee a sufficient data quality for regulatory assessment.

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The benthic isopod Asellus aquaticus as a model biomonitor for metallic nanoparticle pollution in freshwater systems

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Metallic nanoparticles (MNPs) are uniform in size, easy to synthesise, and can be modified to enable binding to ligands or antibodies. They have been defined as "emerging contaminants" and may pose a threat to human life or the environment. There is concern that particles entering the environment may be bioaccumulated by organisms. It is of concern to the environmental scientist that the toxicity of MNPs may be altered by various processes. A biomonitor is a species that gives a measurable response, indicating a change in the surrounding environment. Some species are known to bioaccumulate heavy metal pollutants in the freshwater environment, including the benthic isopod, *Asellus aquaticus*. *A. aquaticus* is tolerant to various types of pollutants, widespread and abundant. The use of *A. aquaticus* as a biomonitor may be an ideal alternative to the current, costly, on-line methods of monitoring pollutants in freshwater systems. As the species bioaccumulates trace metals over its lifetime, it offers the possibility of quantifying a long-term average of otherwise hard-to-detect pollutants, such as MNPs, within the environment, with particular emphasis on bioavailable materials. This could be a crucial step towards implementing a cost-effective biomonitoring programme in locations which may not be easy to access for regular sampling. While a number of studies have considered the effects of pollutants on *A. aquaticus*, few have investigated the processes by which the species ingests, adsorbs, accumulates or excretes the pollutant. There is a great need for further modelling of the fate of MNPs in the environment, and while a model has been proposed for the uptake and accumulation of pollutants by benthic macroinvertebrates, that model does not take into account the process of moulting, as is undergone by *A. aquaticus* every 6-10 days. It has been suggested that moulting may be an adaptation of *A. aquaticus* to expel potentially harmful metals which have accumulated in the carapace, and so any model which is applied to the species must take this process into account. We present a case for the use of *A. aquaticus* as a cost-effective biomonitor for long-term averages of pollutants and a well-adapted method for the monitoring of inaccessible locations. We propose an expanded model for the uptake, excretion and bioaccumulation of metallic nanoparticles in a benthic macroinvertebrate. This model is species-specific, and accounts for the regular moulting of *A. aquaticus*.

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Biodynamics of isotopically labelled CuO nanoparticles and dissolved Cu in the worm T tubifex: Comparing exposure routes

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Upon release into the aquatic environment, metal nanoparticles (NPs), such as CuO NPs will likely agglomerate/aggregate favoring their deposition onto sediments. Very little is known about the biodynamics of uptake of sediment-associated NPs in sediment-dwelling organisms, such as *Tubifex tubifex.* We used the biodynamic model to gain insights into differences in bioaccumulation dynamics between metal forms (aqueous Cu vs CuO NPs) and exposure routes (water vs sediment). The biodynamic model is a simple model that assumes that net bioaccumulation is a result of influx from diet and water and efflux, in the form of physiological loss (k_e) and growth dilution. Isotopically labelled ^{65}Cu was used to avoid confounding influence of background Cu and because it lowers the detection limit for both metal ions and metal NPs. Six different experiments (3 sediment and 3 waterborne exposures) were conducted to parameterize uptake and elimination of $^{65}CuCl_2$ and ^{65}CuO NPs (7 nm) in *T tubifex*. For each exposure route one uptake, one elimination and one net accumulation experiment were conducted for each metal form. Concentrations of newly accumulated ^{65}Cu in samples were determined based on measurements of the naturally occurring stable Cu isotopes (^{63}Cu and ^{65}Cu) using inductively coupled plasma-mass spectrometry (ICP-MS, 7900, Agilent). Our study showed that both $^{65}CuCl_2$ and ^{65}CuO NPs were bioavailable to *T tubifex* regardless of exposure route. Uptake rate constants from water (K_{uw}) was higher for $^{65}CuCl_2$ than for ^{65}CuO NPs (etimated K_{uw}s: 2.5 and 1.2 L g^{-1} d^{-1}, repectively). Similarly, upake rate constants for sediments (K_{us}) were higher for $^{65}CuCl_2$ than for ^{65}CuO NPs (estimated K_{us}s: 0.2 and 0.1, g g^{-1} d^{-1}, respectively), though weight specific body burden were similar for worms exposed to the two Cu forms. 14 d elimination following 3 d water exposures was similar between the two treaments with 59.2±21 and 58.8±17% ^{65}Cu retained for $^{65}CuCl_2$ and ^{65}CuO NPs, respectively (no significant differnce). 21 d of elimination, following sediment exposures was slightly higher for $^{65}CuCl_2$ than ^{65}CuO NPs with 38±18 and 48±9% ^{65}Cu retained, respectively (not significantly different). In summary, there was an overall trend that accumulation of ^{65}Cu in *T tubifex* was higher following exposure to $^{65}CuCl_2$ than ^{65}CuO NPs, especially for water exposures.

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Detection of nano-sized particles in fish following an in vivo dietary exposure: a case study using silver nitrate, silver nanoparticles and silver sulfide nanoparticles
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The toxicity of nanomaterials may correlate with certain characteristics, notably the particle size and the number present in the tissue, emphasising the need for these measurements following exposures. Single particle ICP-MS (spICP-MS) has gained a lot of interest for determining the particle number concentration and particle size distributions in tissues. Here, we present data following an *in vivo* dietary exposure to $AgNO_3$, Ag NPs or Ag_2S NPs. Fish were fed an Ag-supplemented diet (100 mg total Ag/kg) for 4 weeks, followed by 2 weeks depuration. At weeks 2, 4 and 6, the hind intestine, kidney and liver were collected from fish. An alkali based extraction method was developed to remove the particles from the tissue matrix and analyse via spICP-MS. Particulate Ag was found in the hind intestine of all treatments, including the $AgNO_3$ treatment and trace amounts in the controls. At week 4, the particle number concentration (per g dw) in the hind intestine was $7.0 \pm 3.1 \times 10^7$, $31.8 \pm 11.7 \times 10^{10}$, $12.0 \pm 3.3 \times 10^{10}$ and $0.1 \pm 0.0 \times 10^{10}$, for the control, $AgNO_3$, Ag NPs and Ag_2S NPs, respectively. In the Ag treatments, both the $AgNO_3$ and Ag NPs were significantly higher compared to the Ag_2S NP treatment, showing a lower bioavailability of the latter material. Additionally, this indicates that particulate Ag can be made in either the gut lumen or within the intestinal tissue. There was no difference in the median particle size of the hind intestine or kidney of any Ag treatment (30-50 nm), suggesting the particles released from the intestine and circulate in the blood to other organs. Interestingly, in the liver, the median particle size of the $AgNO_3$ and Ag NP treatments significantly increase to around 80 nm compared to the controls (50-60 nm). Such increases in particle size suggests an agglomerative process or more complex ionic deposition to particles. In conclusion, fish can be exposed to particles from the gut lumen or tissue, which are subsequently released from the hind intestine into the circulatory system and enter other organs where particle transformation processes may (liver) or may not (kidney) occur. To support this spICP-MS data, whole organ proteome of the hind intestine and liver will be presented to determine sublethal toxicity. Additionally, these protein expression profiles will potentially inform on the form of Ag entering the intestine. This work was supported by an EC funded grant, NanoFASE (number 646002).

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Removal of arsenic from contaminated seawater by manganese spinel ferrite nanoparticles: ecotoxicological evaluation in Mytilus galloprovincialis
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In recent years, nanotechnology has developed new materials with several advantages in relation to traditional water treatment methods. For example, manganese-ferrite ($MnFe_2O_4$) nanoparticles (NPs) have a large specific surface area and many active sites, being very efficient sorbents for different potentially toxic elements in contaminated waters, including arsenic (As) that is currently listed as the top-priority hazardous substance by the Agency for Toxic Substances and Disease Registry. Moreover, due to its magnetic properties, $MnFe_2O_4$ NPs can be easily separated from water after treatment by applying a magnetic field. Recently, we optimized the synthesis of these NPs (and operational conditions) demonstrating that a small amount can reduce significantly the levels of As in contaminated water (>90% removal). In the present study we aimed to evaluate the ecotoxicological safety of the remediated water, which is common lack in most of the works. Mussels *Mytilus galloprovincialis*, which have been identified by several authors as a good bioindicator species that responds quickly to environmental disturbances, with spatial distribution and economic relevance were chosen as species model. Organisms were exposed for 28 days at 17ºC and salinity 30 to different conditions, including clean seawater (control); remediated seawater; As (1000 μg/L), $MnFe_2O_4$ NPs (50 mg/L) and mixture of both, with weekly water renewal. At the end of exposure, concentrations of As in mussels tissues were quantified and biomarkers related to mussels' metabolic and oxidative stress status were evaluated. Results revealed that mussels exposed to water contaminated with As and to As+NPs accumulated significantly more As than those exposed to the remediated seawater. Regarding biomarkers, our findings demonstrated that contaminated seawater clearly caused higher impacts in mussels than decontaminated seawater, with higher oxidative stress and lower metabolic capacity in mussels exposed to contaminated seawater.

Advances in Soil Ecotoxicology and Risk Assessment - Impact, Ecotoxicity Tests, and Concepts for a Retrospective Environmental Risk Assessment (II)

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Effects of climate-related stressors on survival, reproduction and physiological state of the earthworm Eisenia andrei
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General estimations point to 1 billion hectares of salt affected areas and this number is expected to increase. Besides, the expected changes in precipitation regimes and the registered increase of 0.85ºC on surface temperature observed between 1880 and 2012, will probably enhance extreme events. A few studies on the effects of salinity or temperature and/or moisture on soil ecosystems have been conducted but their simultaneous effects on soil invertebrates has not yet been assessed. The present study aimed at evaluating the effects of three climate-related stressors, namely salinization, temperature and moisture, on the survival, reproduction and physiological state of the earthworm *E. andrei*. To fulfill such aim, the ISO earthworm reproduction test was performed to assess survival and reproduction, and a parallel 28-day test was carried out to assess the effect of above-mentioned stressors on the available energy reserves (*Ea*), i.e. lipid, carbohydrate and protein contents, and energy consumption measured at both the cellular level (*Ec*) and as the whole-organism respiration rate (*R*). A total of 27 treatments were tested, which included three levels of salinity (0, 24 and 31% of seawater), moisture (35, 50 and 70% of the water holding capacity, WHC), and temperature (20, 25 and 30ºC), in a full factorial design. Results showed that all three stressors, and their interactions influenced life-cycle parameters of the earthworms. Both salinity levels decreased survival at both 35 and 70% WHC and at all temperatures with the strongest effect at 30ºC. Similarly, both salinity levels decreased reproduction with the strongest effect found at 35% WHC. The reproduction was the lowest at 30ºC, but negative effect of salinity was found at all temperatures. The high mortality of earthworms at 35% WHC and 31% of seawater did not allow for testing the effects of all possible interactions between stressors on physiological endpoints. Nevertheless, decreased *R*in earthworms exposed for 28 days to 24% of seawater at 70% WHC was found at 20 and 25ºC. Coping with stressors,a decrease in energy reserves was expected. However, no significant impact on any component of *Ea*was found in those individuals which survived 28 days of exposure, probably due to different allocation of energy: the earthworms from lower-stress conditions reproduced, but those exposed to higher-stress conditions might use energy to meet the increased energetic demand to survive.

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Do climate factors affect invertebrates' avoidance behaviour in anthropogenic-degraded soils?
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The Intergovernmental Panel on Climate Change (IPCC) predicts major changes in future climate conditions. Soils will be among the most affected components of terrestrial ecosystems, especially in anthropogenic-degraded areas where soil biota has to deal with already multiple stressors. This study aimed at assessing the effects of different climate factors (soil moisture content, air temperature, ultraviolet radiation and atmospheric CO_2) on the avoidance behaviour of two invertebrate species (*Folsomia candida* and *Enchytraeus crypticus*) in anthropogenic metal(loid)-contaminated soils. Two soils degraded by metal(loid) contamination were selected in central-northern Portugal: mining soil; former agricultural soil near an industrial chemical complex. Lufa 2.2 soil was used as control soil. The avoidance behaviour of *F. candida* and *E. crypticus* was evaluated under different climate conditions: 20 ºC + 50% soil water holding capacity –WHC– (standard climate conditions); 20 ºC + 75% soil WHC; 20 ºC + 25% soil WHC; 15 – 25 ºC + 50% soil WHC; 20 – 30 ºC + 50% soil WHC; 25 – 35 ºC + 50% soil WHC; 20 ºC + 50% soil WHC + UV radiation (UVR) exposure; 20 ºC + 50% soil WHC + 600 ppm atm CO_2; 20 ºC + 50% soil WHC + 800 ppm atm CO_2; 20 ºC + 50% soil WHC + 1000 ppm atm CO_2. Both invertebrate species had different avoidance response towards the contaminated soils whether under standard climate conditions or when individual climate factors were changed. Under standard climate conditions *F. candida* showed a homogeneous distribution between the mining and control soils and a slight preference for the agricultural soil, while *E. crypticus* avoided both contaminated soils. When changing the

climate conditions *F. candida* maintained the same behaviour, except when exposed to higher atmospheric CO_2 levels in which organisms avoided metal(loid)-contamination. *E. crypticus* avoided both contaminated soils when changing the different climate factors evaluated, except when exposed to high (75% soil WHC) and low (25% soil WHC) soil moisture content. This study showed that both invertebrate species differed in their capacity to avoid anthropogenic metal(loid)-contaminated soils and that changing climate conditions may affect their avoidance response. *F. candida* only avoided metal(loid) contamination when exposed to high levels of atmospheric CO_2, while *E. crypticus* lost their capacity to avoid metal(loid)-contaminated soils when exposed to flooding and drought soil conditions.

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Microbial communities in As contaminated agricultural soils -How PK fertiliser can influence As speciation

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In 2018, 220 million people are still exposed to As from drinking water or food. When crops are grown on arsenic-rich soils, or irrigated with arsenic-containing water, agricultural products, such as rice, corn or barley can contain high arsenic levels, and the bio-accumulation may be linked to arsenic speciation. The present work, performed as part of the WaterJPI AgriAs project, focuses on the links between As concentration and speciation in soils, soil redox conditions and fertilization (PK) through the application of a range of microbial bio-indicators. In soils contaminated contaminated with As from the destruction of WWI weapons, soil microbial communities and abiotic parameters were measured. Soils were then incubated in oxic or anoxic conditions with increasing amounts of PK fertilizer to assess its impact on the fate of As. As concentrations on-site affect microbial communities with higher abundances of As-oxidising and reducing bacteria in the most contaminated soil. A PK amendment in oxic conditions favoured As speciation as AsV, as expected, and only reduced its mobility for the highest PK amounts, possibly due to mineral precipitation. In anoxic conditions however, the PK amendment significantly reduced As mobility although most of the As was AsIII. This could also be linked to mineral precipitations of phosphorus with As. These results are aimed to help provide recommendations to the EU for management of As-contaminated soils that present a risk to populations.

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A lab-to-field approach to monitor the exposure of soil microbial communities to β-triketone herbicides

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β-triketone herbicides are among the most used herbicides in corn crop. These herbicides inhibit the 4-hydroxyphenylpyruvate dioxygenase (4-HPPD), lead to bleaching and prevent the growth of broadleaf weeds. This enzyme is not only found in plants but in all living organisms, including microorganisms where it plays a role in the tyrosine degradation pathway. Thus, microorganisms classified as “non-target organisms” by current EU regulation for pesticide authorization, might be impacted by β-triketones, with consequences on microbial function supporting soil ecosystem services. Since microorganisms have been proposed by EFSA as key-drivers to protect soil ecosystem services, we suggest a novel approach consisting in considering the *hppd* bacterial community as a biomarker sensitive to the exposure to β-triketone residues. With this objective, we developed a toolbox to monitor the abundance, the composition, the diversity and the activity of the *hppd* bacterial community. Each method was tested in a lab-to-field experimental design following the tiered-approach recommended by EFSA to conduct pesticide environmental risk assessment (ERA). Under lab conditions, soil microcosms not exposed (control) or exposed to x1 or x10 the agronomical dose of sulcotrione (active ingredient) or Decano® (one of the commercial formulation of sulcotrione) were studied. Under field conditions, samples were collected in corn crop exposed to β-triketones. Soil samples were also collected from a non-treated corn field (not exposed control). Analytical chemistry was applied to all samples to search for β-triketone residues and to estimate the scenario of exposure of soil microorganisms. Nucleic acids (DNA/RNA) were extracted from soil samples to measure the abundance (quantitative PCR), the expression (quantitative RT-qPCR), the composition (α-diversity) and the diversity (β-diversity) (NGS) of the *hppd* bacterial community. Our results will be presented to the audience with the aim to identify the better proxy of the *hppd* bacterial community that could be used as a biomarker to reflect the exposure of soil microbial community to β-triketone residues. This explanatory work might be extended to other pesticides targeting other enzymes that are also present in so call non-target organisms such as sulfonylureas inhibiting acetohydroxy acid synthase (AHAS).

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Efflux pump activity in Enchytraeidae

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Enchytraeids are ecologically relevant soil organisms, which play an important role in organic matter decomposition and soil bioturbation. They live in close contact with the pore water fraction of soil which makes them vulnerable to xenobiotics exposure. MXR proteins (efflux pump) transfer certain groups of xenobiotics out of the cell and keep the intracellular concentration of these compounds low. They are present in all organisms, including microbes, plants, invertebrates and vertebrates. In the phylum Annelida MXR activity was discovered mainly in earthworm species but none enchytraeids. For the identification of MXR system activity fluorescent substrates are often used. In case of MXR system inhibition, the concentration of the substrate in the cells increases, and this can be seen as a rise in fluorescence. Enchytraeids were exposed in distilled and ISO test water, with or without specific inhibitors and inducers at various concentrations for 1 h in the dark. After pretreatment they were exposed to various concentrations of Rhodamine B (RhB) or Rhodamine 123 (Rh123). The measurement of RhB and Rh123 amount in samples were conducted immediately after centrifugation. The samples were kept in dark due to the photo degradation of RhB and Rh123. The amount of RhB and Rh123 in the samples was measured using spectoflourometer. Using RhB and R123 as the fluorescent substrate, it could be seen that different substrates and chemosensitizers have an effect on the amount of fluorescence in the *E. albidus* and *E. crypticus*. Some metal nanoparticles and pesticides are ubiquitous environmental toxicants and potential MXR modulators that pose a continuing risk to many non-target species. The ability of enchytraeids to remove the xenobiotics from the cells will greatly impact the final harmful effects. Determining presence and function of the MXR in enchytraeids can help us to understand the consequences of MXR inhibition at the population and ecosystem level. This study shows that enchytraeids, as a model species in terrestrial ecotoxicology, possess MXR activity and that different substrates and chemosensitizers have an impact on it. Therefore, it can be presumed that xenobiotics present in the environment will have an effect on MXR system as well. Due to the influence of MXR activity on bioaccumulation and toxicity of various compounds, the assessment of MXR activity could be a valuable complementary biomarker in ecotoxicological research and risk assessment.

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Earthworm avoidance behavior quantification using artificial neural networks

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Avoidance behavior of earthworms represents a valuable endpoint in ecotoxicological studies examining the\neffects of sub-lethal concentrations of pollutants on soil-dwelling fauna. Since it was first performed by\nYeardley (1996), the earthworm avoidance test was proven to give ecologically relevant insights into modes\nof action of various pollutants. Since behavioral responses of test organisms are highly variable over time,\ndevelopment of techniques that would allow continuous monitoring of avoidance behavior are of great\nimportance.\nIn this research, a novel approach is presented towards developing a low-cost, automated monitoring method\nfor continuous examination of earthworm behavior within avoidance tests. The suggested system\nimplements a 2D terrarium, a tool widely used in earthworm behavioral studies, as a compartment in which\nthe avoidance test is performed. Automatization of avoidance behavior detection analysis is achieved using a\ndeep convolutional neural network model, constructed and trained in Keras, that gives precise predictions of\nearthworm locations in images of both sides of the terrarium during the test period. To quantify the motion of\nthe experimental organisms in 2D space, the area of the terrarium is divided into multiple segments.\nPerformance of the presented system was tested in avoidance tests performed in glass terrarium filled with\nclean artificial soil in one half and artificial soil spiked with oil mill waste in the other half. Predictions of\nearthworm location probabilities over the image-pairs show high level of overlap, complementing each other\nand increasing certainty of automatic earthworm location determination. The first period of the experiment is\ncharacterized by equal presence of earthworms in both halves of the terrarium, with a gradual shift from the\nbottom to the top layers. Thereafter, a transition to the unpolluted (control) half is observed. This period of\nthe experiment is characterized by the localization of earthworms in the bottom half of the control soil.\nThe presented ANN-based approach proved to be useful in continuous monitoring of earthworm behavior.\nDue to its low cost and simple design, it is an easily reproducible setup that gives a significant acceleration to\nanalysis and interpretation of data resulting from behavioral studies, with the potential of becoming an ultimate\ntool in research of primary modes of action of various pollutants present in the environment.

Adverse Effects of Chemicals on Host-associated and Free-living Microbiomes (I)

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Effects of microcystin and extracts from toxin-producing cyanobacterial strains on the gut microbiota of medaka fishes (Oryzias latipes) : a microcosm approach

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The importance of animal-associated microorganisms in various aspects of host biology is increasingly recognized, and contributes innovative lines of research in various areas of life sciences. Sit at the interface between an organism and its environment, the microbiota must be accounted for in ecotoxicology studies, as much as the host organism itself. The effect of toxin-producing cyanobacterial blooms on fishes health has been extensively investigated, yet no study to date has evaluated this effect of fish-associated microbiota. In this first effort to document this effect, we exposed a model fish, the medaka *Oryzias latipes*, to pure microcystins and to crude extracts of metabolites from cyanobacterial cultures in order to test their influence on microbiote composition. Histology approaches were used to estimate the impact of toxins on fish liver and to evaluate the density of bacterial communities present on the gill, skin and gut of fishes. Metabarcoding approaches using the 16S rRNA-encoding gene were used to investigate bacterial community composition at the individuals level. We show that fish gut harbors the highest bacterial densities. Gut-associated communities differ from those in the water, and among individual fishes. Exposure to extracts, rather than pure microcystin, has a significant influence on gut community compositions. We suggest that compounds present in the cyanobacterial extracts, but not microcystin alone, alter the composition of bacterial communities, with possible consequences for the digestive function of fishes. Based on this very simple microcosm setup, we propose that the effect of cyanobacterial blooms on fish gut microbiota and associated functions, including toxin degradation and feed efficiency, should be further explored.

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Effect of triclosan on benthic microbial communities in aquatic ecosystems: a microcosm study

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Aquatic ecosystems receive a wide range of pollutants such as pesticides, pharmaceuticals and personal care products. One of the pollutants that can be found in aquatic ecosystems is triclosan, a synthetic antibacterial compound which inhibits the enzyme enoyl-ACP reductase, essential in the bacterial fatty acid biosynthetic pathway. Due to its antimicrobial properties, triclosan may negatively affect the abundance, diversity and activity of benthic microorganisms. Since microorganisms are essential for aquatic ecosystems, carrying out crucial ecosystem functions such as decomposition, nutrient cycling and primary production, it is pivotal to understand the pollutant-induced effects for the protection of those systems. We used a microcosm approach to assess the effects of triclosan on benthic microbial communities of natural and artificial sediment in situ colonized by microorganisms of two different waterbodies. More specifically, we (1) evaluated how microbial communities respond to exposure of triclosan, (2) assessed whether those responses differ between waterbodies and (3) evaluated whether we can use in situ colonized artificial OECD sediment in the assessment of pollutants on microbial communities. We exposed microcosms to different triclosan concentrations for 7 days in the laboratory. These microcosms contained natural sediment collected from two non-contaminated waterbodies and artificial sediment inoculated at those two sites. HiSeq sequencing of the V4 region of archaeal and bacterial 16SrRNA gene was used to characterize the microbial communities for the different treatments. Initial results of the microcosms containing artificial sediment inoculated in a ditch do not show clear effects of triclosan on microbial diversity and community structure. However, in addition we will analyse the samples by qPCR to determine the abundance of total bacteria (16S rRNA gene) and analyse the other three sediment treatments.

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The effects of biocidal nanoparticles on the midgut bacterial community and immune responses in earthworms

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A major application of nanoparticles (NP) is as an antimicrobial agent in paints, coatings and pesticides. As the microbiome of animals can play an essential role in the health of their host, the release of biocidal NP into the ecosystem might lead to adverse health effects in animals should their microbiome be negatively affected by exposure to a biocide. This paper studies the impact of biocidal metal NPs on the bacterial community composition in soil and the midgut of the earthworm *Eisenia fetida*. Additionally, host immune responses to a biotic challenge under NP exposure are also assessed in order to study the implications of sublethal NP exposure on host health. To study the impact of biocidal NPs on the midgut bacterial community, two 28-days long exposures were conducted, one using copper oxide NP, the other using silver NP. At the end of the exposure, the community composition in the soil and the earthworm midgut was assessed by amplification and sequencing of the 16S-rRNA gene. To study host immune responses under sublethal NP exposure, earthworms were exposed to either copper oxide NP, ionic copper or negative control for 28 days and subsequently exposed to either a high dose of *Bacillus subtilis* or a negative control before returning to the original exposure soils for recovery. Five sampling points were selected throughout the experiment. At each sampling point, the bacterial community composition of the soil and the earthworm midgut was assessed as well as the expression of various immune genes in earthworm midgut tissue and coelomic fluid. The data show that copper NPs cause a significant shift in the bacterial community composition in the soil and the earthworm midgut. The most dominant core midgut taxa are hardly affected by the exposure. However, the relative abundance of certain core taxa including *Candidatus* Lumbricinola and a *Spirochaetacaea* are significantly reduced under copper exposure. Exposure to silver NP caused a strong shift in the soil bacterial community but no significant effect of silver NP on the midgut bacterial community was recorded. All samples from the experiment assessing host immune responses to a bacterial challenge under NP exposure have been collected and results will be included in the presentation. This study shows that biocidal metal NP can induce community changes in microbiome of earthworms with the possible loss of some core bacterial taxa at copper levels frequently reported in agricultural soils.

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Multi-omics approach to determine the effect of bis(2-ethylhexyl) phthalate on microbiome-gut axis

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Phthalate esters, such as DEHP (bis(2-ethylhexyl) phthalate, are a ubiquitous class of chemicals used as plasticizers in plastic products such as toys and hospital supplies. They are not covalently bound to the polymer matrix and can leach out of products. Experimental studies suggest that microbiome and gut play significant role in metabolism and disruption of microbiome-gut axis leads to effects on intake of nutrient, hormone balance, immunity and nervous system. The goal of this study is to determine whether DEHP, a model metabolic disruptor, adversely affect the zebrafish (*Danio rerio*) microbiome-gut axis and describe how taxonomical change in microbiome are reflected in gut. We exposed the fish to 3 ppm DEHP via food for 8 weeks to stimulate chronic exposure. Fish were fed once a day with commercial food mixed with DEHP. Fish were dissected and the content of gut and the gut tissue were collected and flesh frozen. The composite and diversity of microbiome were studied by analyzing of 16s RNA genes using Illumina MiSeq. Subsequently, we link the phylogeny and the function of the microbiome using a bioinformatics approaches. To identify genomic responses of DEHP in gut, we used microarray and real-time RT-PCR analysis followed by differential gene expression analysis. The data were then mapped to changes in cell processes and signaling pathways using Pathway Studio. This study revealed, that DEHP can significantly effects the composition of microbiome and its related function, and that these microbial changes together with the direct effect on gut has an effect on gut's transcriptome. Interestingly, the changes on gut and microbome are different for both sexes. The aim of this presentation is to present the microbiome-gut axis as a target for metabolic disruptors, such as DEHP and emphasize the need of multi-omics approach to study this phenomenon. This research was supported by Marie Sklodowska-Curie actions no.707241, by CETOCOEN PLUS (CZ.02.1.01/0.0/0.0/15_003/0000469) and RECETOX RI (LM2015051).

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Early-life exposure alters copper tolerance in the stickleback gill microbiome

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The microbiome influences host phenotype in many ways. While stress-disruption of the microbiome may induce adverse health effects on host health, metagenomic plasticity may represent a potential mechanism of stress-tolerance in natural fish populations. We investigated the effect of copper, a widespread environmental pollutant, on the gill microbiome of the three-spined stickleback (*Gasterosteus aculeatus*). We aimed to characterise the impact of environmentally-relevant

concentrations of copper on the gill microbiome of adult stickleback, and also to investigate whether pre-exposure to copper in early life affected later-life microbiome copper-tolerance. We exposed stickleback embryos/larvae to either 0 or 10 μg/L copper from 1-217 hpf, grew fish to sexual maturity under control conditions, and then re-exposed adult fish from each group to either 0, 10 or 20 μg/L copper for four days. Gill microbial community profiling was performed using 16S rRNA amplicon sequencing (V4 region), with OTUs assembled and annotated using mothur and Silva reference data. We found that adult exposure to copper had little impact on gill microbial Chao1 richness or Shannon diversity, but did have a clear effect on microbiome structure. Exposure to 20 μg/L copper induced a significant change in community structure relative to control (unexposed) fish, regardless of pre-exposure. This change was characterised by a significant increase in the abundance of several OTUs with known copper tolerance, including *Deinococcus* sp., and also induced a marked change in predicted community function. In contrast, the effects of exposure to 10 μg/L copper were strongly influenced by pre-exposure in early life. While fish that were not pre-exposed to copper showed similar structural and functional changes to those identified in the higher copper treatment group (20 μg/L), the fish that had been pre-exposed to copper displayed gill microbiome structure more similar to the control (unexposed fish). Overall, we have shown that copper exposure induces structural and functional changes in the gill microbiome, and these may potentially also influence host phenotype. However, our results also suggest that pre-exposure to copper during early development can increase microbiome copper-tolerance in later life which may, potentially, also influence host tolerance of copper.

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The Microbiome Stress Project: towards a global meta-analysis of environmental stressors and their effects on microbial communities

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Microbial community structure is highly sensitive to natural (e.g. drought, temperature, fire) and anthropogenic (e.g. heavy metal exposure, land-use change) stressors. However, despite an immense amount of data generated, systematic, cross-environment analyses of microbiome responses to multiple disturbances are lacking. Here, we present the Microbiome Stress Project, an open-access database of environmental and host-associated 16S rRNA amplicon sequencing studies collected to facilitate cross-study analyses of microbiome responses to stressors. This database will comprise published and unpublished datasets re-processed from the raw sequences into exact sequence variants using our standardized computational pipeline. Our database will provide insight into general response patterns of microbiome diversity, structure, and stability to environmental stressors. It will also enable the identification of cross-study associations between single or multiple stressors and specific microbial clades. Here, we present a proof-of-concept meta-analysis of 606 microbiomes (from nine studies) to assess microbial community responses to: (1) one stressor in one environment: soil warming across a variety of soil types, (2) a range of stressors in one environment: soil microbiome responses to a comprehensive set of stressors (incl. temperature, diesel, antibiotics, land use change, drought, and heavy metals), (3) one stressor across a range of environments: copper exposure effects on soil, sediment, activated-sludge reactors, and gut environments, and (4) the general trends of microbiome stressor responses. Overall, we found that stressor exposure significantly decreases microbiome alpha diversity and increases beta diversity (community dispersion) across a range of environments and stressor types. We observed a hump-shaped relationship between microbial community resistance to stressors (i.e. the average pairwise similarity score between the control and stressed communities) and alpha diversity. We used Phylofactor to identify microbial clades and individual taxa as potential bioindicators of copper contamination across different environments. Using standardized computational and statistical methods, the Microbiome Stress Project will leverage thousands of existing datasets to build a general framework for how microbial communities respond to environmental stress

Novel Developments in Testing Chemicals for Endocrine Disrupting Properties (I)

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Cross-species Applicability of a Quantitative AOP Describing Inhibition of Aromatase Activity Leading to Reproductive Dysfunction in Fishes

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Quantitative adverse outcome pathways (qAOPs) describe quantitative response-response relationships linking the molecular initiating event (MIE) and adverse outcome (AO) to enable quantitative prediction of the probability of occurrence or severity of an AO for a given magnitude of chemical interaction with an MIE. A qAOP has been developed for inhibition of cytochrome P450 aromatase (CYP19) leading to reproductive dysfunction through decreased circulating estradiol (E2) thereby reducing circulating vitellogenin (VTG). This qAOP was developed based on quantitative data from the fathead minnow (*Pimephales promelas*). However, whether a qAOP developed based on data from fathead minnow could predict reproductive dysfunction in other fishes was unknown. Therefore, this study investigated whether this qAOP could accurately predict adverse responses to the model CYP19 inhibitor fadrozole for three other fishes, namely Japanese medaka (*Oryzias latipes*), zebrafish (*Danio rerio*), and mosquitofish (*Gambusia affinis*). Japanese medaka and zebrafish have asynchronous oocyte development in common with fathead minnow. In contrast, mosquitofish have group-synchronous oocyte development which is the prominent strategy among fishes. *In vitro* CYP19 inhibition assays demonstrate comparable sensitivities to fadrozole among fathead minnow, Japanese medaka, and zebrafish, while CYP19 of mosquitofish is 13-fold more sensitive. Results of 21-day reproductive assays demonstrate comparable quantitative response-response relationships describing fadrozole to E2, E2 to VTG, and VTG to egg production among the asynchronous fishes. However, mosquitofish have complex profiles of sex steroids and steroidogenic enzymes characteristic of group-synchronous oocyte development and exposure to fadrozole causes compensatory responses unique from asynchronous fishes. Overall, these results suggest that the qAOP developed for fathead minnow can broadly predict reproductive dysfunction among fishes with asynchronous oocyte development, which includes numerous small-bodied fishes. But, fishes with group-synchronous oocyte development have different compensatory responses and other modeling challenges which require further investigation towards development of an applicable qAOP. The results of this study could be essential in guiding more objective ecological risk assessments of fishes to aromatase inhibiting chemicals.

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The direct link between endocrine MoA and adverse effect - Concepts to integrate molecular information in endocrine disruptor testing

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To be defined as endocrine disruptor, a substance has to meet several criteria, including the induction of specific adverse effects, a specific mode of action (MoA), and a plausible link between both. In order to prove causality, we applied different approaches, which utilize the Adverse Outcome Pathway (AOP) concept. We demonstrated that AOPs, in this case the AOP for Aromatase inhibition leading to reproductive dysfunction in fish, can be transferred to other life stages, and also other species. Indentical molecular initiating events (MIEs) and key events (KEs), as well as their relationships (KERs) are responsible for specific effects in different life stages. Thus, similar screening methods might be applied for the prediction of different, endocrine-related adverse effects, like reproduction in adult fathead minnow and a skewed sex ratio in juvenile zebrafish. Presumably sexual endocrine-related effects might also be triggered by other MIEs, belonging for example to endocrine and non-endocrine AOPs. Identification of these differences is of mayor importance, as regulation of endocrine disruptors is based on hazard assessment, while for non-endocrine substances a risk assessment has to be performed. Tests with fish performed according to OECD test guidelines are often noch able to identify the mode aof action of a sbustance, as only apical endpoints, which could be triggered by substances with different MoAs, are assessed. AOPs for these different MIEs are existing; however, the data gap has to be closed by methods not routinely applied in risk and hazard assessment. For example molecular signatures, which are MIE-specific rahter than substance-süecific, can be identified by high-content approaches like proteomics or RNAseq. We applied this approach in order to discriminate the AOP of aromatase inhibition form the AOP of hepatotoxicity during the reproductive pahse of adult zebrafish. Already the number of regulated proteins/genes in the respective tissues can give hints to the most affected organ. Furthermore, a deeper liik into the signaling pathways and into specific regulated proteins allow the identification of biomarkers, which can be further developed to be applied in *in vitro* screening tests, e.g. with zebrafish embryos.

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Ambient temperature modulates biological effects of estrogenic exposure in

freshwater fish

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In ectothermic organisms, growth, reproduction, and behavior are mediated by changes in ambient temperature. Because the molecular mechanisms underlying such biological rhythms are closely integrated with the hypothalamus-pituitary-gonadal axis, environmental signals such as ambient temperature may modulate the effects of endocrine disrupting compounds in aquatic environments. In a series of four month-long experiments, we exposed larval and adult fathead minnows to three concentrations of estrone (E1) at four temperatures experienced during the breeding season (15, 18, 21, 24ºC). E1 concentrations represented ecologically relevant (E1-L 14ng/L; E1-M 22ng/L) or worst-case environmental scenarios (E1-H 65ng/L estrone). Larval fish were assessed for survival, growth and performance in predator avoidance and foraging assays, respectively. Reproductive, behavioral, morphometric, and physiological endpoints were tracked in adult fish maintained in breeding groups of one male and two females. We observed significant interactions between E1 concentration and temperature that influenced larval escape velocity and foraging performance, especially at lower temperatures. Adult survival, clutch size, and female morphological endpoints also experienced temperature modulation of E1 exposure effects. Our data suggest that the effects of contaminants can vary with thermal regime, especially at colder temperatures and in female fish. In addition, earlier life stages are more susceptible to the modulating effects of temperature on anthropogenic stressors. This study adds evidence that even under constant conditions that diverge from standard laboratory protocols, the expression of biomarkers consistent with exposure to endocrine disrupting compounds may change.

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Endocrine potential of a small German stream influenced by a large WWTP - a combined assessment with cell-based and organism-based methods

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Not all European surface waters will reach the goal of the European Water Framework until 2027. One reason might be the release of a variety of anthropogenic micropollutants (MPs). They could be released via point sources as waste water treatment plants (WWTPs). Not all MPs are completely eliminated in the conventional treatment process and thus enter the aquatic environment. The implementation of a further treatment step, e.g. ozonation, could reduce this burden. A commercial scale ozonation plant was implemented at the “Aachen Soers” WWTP in 2017. It releases its effluent into the stream Wurm. To elucidate the impact of the additional treatment step, the *status quo* was recorded before and after the implementation. Beside numerous endpoints one important endpoint is the endocrine activity, which was evaluated by different CALUX® assays ((anti-)estrogenic, (anti-)androgenic), and by the reproduction assay with *Potamopyrgus antipodarum*. Samples up- and downstream the WWTP and the WWTP outlet were assessed. Results before upgrading have shown a reduced estrogenic potential downstream compared to upstream the WWTP. In contrast, there was a reduced embryo number upstream. There is no clear explanation, except the reduced measured temperature. Additionally, the embryo number was increased downstream compared to samples upstream and to the control. The temperature downstream the WWTP outlet was similar to the laboratory control, hence an effect by MPs could be most reasonable. But, also other environmental factors might have an influence. Anti-androgenic activity was measured at all sampling sites before upgrading. Anti-estrogenic activity was slightly higher downstream the WWTP. First results after implementation of the WWTP are available and will be finished until May 2019. Up to now, the study has shown, that cell-based bioassays are a good screening tool for endocrine activity, but also environmental factors can have a high impact on effects in organisms. Combining cell-based and organisms-based bioassays is a good choice to get a comprehensive picture of the environmental situation. Additionally, it is also necessary to measure not only the estrogenic but also other endocrine potentials to get a full picture of the chemical impact. *Acknowledgement* - The authors thanks the German Ministry for Climate Protection, Environment, Agriculture, Conservation and Consumer Protection of North Rhine-Westphalia for funding the DemO$_3$AC -project.

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Breaking down the wall between human health and environmental testing of endocrine disrupters EndocRine Guideline Optimisation (ERGO)

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The authors present a new approach that will support a paradigm shift in the regulatory use of standardized test guidelines (TGs) by breaking the existing wall between mammalian and non-mammalian vertebrate testing and assessment of endocrine disrupting chemicals (EDCs). The highly conserved thyroid system will be used as the “proof of concept”, but also other conserved endocrine axes/systems such as the Retinoid X Receptor (RXR) and the Hypothalamus Pituitary Gonadal (HPG) axis can be adapted to the cross-vertebrate class approach. The project, called ERGO, will investigate a battery of draft in vitro assays and evaluate thyroid-responsive biomarkers and endpoints suitable for extrapolation of effects from fish and amphibian tests to humans and other mammals (and vice versa) and finally validate successful biomarkers and endpoints for inclusion in existing in vivo or new in vitro OECD TGs. A cross-class adverse outcome pathway (AOP) network will provide the scientifically plausible and evidence-based foundation for the selection of biomarkers and endpoints in lower vertebrate assays predictive of human health outcomes. In silico modeling and biotransformation data will support cross-vertebrate class effect extrapolation. Here we present the background and expected major outcomes of ERGO. An EDC project Cluster consisting of ERGO and 7 sister projects funded under EU Horizon2020 will also be presented and discussed. This Cluster represents the largest investment in EDC research ever funded in Europe and runs from 2019-2024.

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Using population modelling to assess population relevance of endocrine-mediated effects of plant protection products in non-target vertebrates

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European Commission´s hazard-based criteria to identify the endocrine disrupting properties of plant protection products (PPPs) applied across the European Union from 10th November 2018 (EC 2018). The implementation of this approach is supported by a Guidance Document (ECHA/EFSA 2018). For non-target organisms, a PPP active substance that meets the criteria is considered as having endocrine disrupting properties unless there is evidence demonstrating that the ED-related adverse effects identified are not relevant at the population level. According to EC (2018), the evaluation of population-relevant effects shall consider adequate, reliable and representative field or monitoring data and/or results from population models where available. However, in the Guidance Document, EFSA/ECHA considers that setting general rules for the evaluation of field studies and monitoring data is complicated, resulting in case-by-case evaluations. EFSA/ECHA acknowledges the existence of different population models that can be used for different purposes and for answering different questions such as the degree of reproductive impairment which is likely to trigger consequences at the population level. However, the Guidance Document does not provide recommendations on how population models should be used to assess population-relevant adversity in a hazard-based assessment. In this presentation, we describe a generic and modular modelling framework which complement this Guidance and would assist registrants and regulatory authorities to identify substances for which a potential endocrine disruption issue could be concluded from laboratory toxicity tests, but which would not translate into adverse effects on wildlife populations. The concept of Maximum Tolerated Concentration/Dose (MTC/D) and the concept of focal species are addressed, and we explain the conditions in which Dynamic Energy Budget (DEB) models can be combined to Individual-Based Models (IBM) without accounting for individual exposure. \n

Improving Decision Support for Sustainability Going beyond Standard LCA (I)

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Closer than ever to seamless exchange of data and models between LCA software tools: lessons learned and improvement opportunities from the EF Remodeling project

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Similar data and methods are implemented in various LCA software (SW) tools, however there are cases of the same dataset generating different results in different tools. Also, users tend to use one SW tool and when they want to move their data or model to another SW, they face many obstacles. In the context of the Environmental Footprint (EF) pilot phase, the European Commission requested the development of 63 representative products/organisations (RPs/ROs), which: are consistent with the requirements of the final product environmental footprint category rules (PEFCRs) and organization environmental footprint sector rules (OEFSRs), use the prescribed EF-compliant secondary datasets, and can be made available for free to any user applying a PEFCR/OEFSR and are easily usable in the major existing LCA SW tools. An implicit prerequisite is that SW tools calculate equal results for the same RPs/ROs. The remodeling project included five software tools: GaBi, openLCA, RangeLCA, SimaPro, and Umberto. 59 aggregated datasets, one for each representative product, developed in the ILCD format, were tested in all five SW tools. Already for a rather simple task, i.e. to calculate LCIA results for one aggregated dataset, a few iterations were needed – to align on flow mappings, LCIA method implementation, etc. – but ultimately all five SW tools calculated LCIA results with a difference lower than 1%. Because the ILCD format is limited to single process datasets, we developed a life cycle model (eILCD) format aimed at facilitating model exchange among the tools. Seven models, developed in four SW tools, were tested by all partners. Some models could be successfully exchanged between SWs and a few provided equal LCIA results, but not all of them. This presentation will cove: the problems faced when importing the models and investigating the sources of differences in the results in the different SW tools, as well as suggestions on how to address the import problems and results differences. This work is the basis to enable the reduction of software-system related issues. We believe this is a crucial step towards the exchange of data and models across SW tools and the comparability of results. We are definitely closer to that than we have ever been before… so we can't stop now. We need to capitalize on the work done and on lessons learned during the Remodeling project and improve what we have so far to ultimately make data and model exchange truly possible for all users.

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Incorporation of hybridised impact assessment methods into LCA for the environmental evaluation of bio-based products

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Adoption of bio-based products has been identified as one of the most promising pathways to establishing a sustainable and fully-functional bioeconomy. With an ever-increasing demand for bio-based products in the commercial market and public perception of instinctively associating bio-based products to being "sustainable" from cradle-grave, there is now a need for the sustainability communities to identify ways of mapping the threats and opportunities embedded in a given product and the process. The aim of this study is to propose and test the effectiveness of methodologies that incorporate the principles of sustainable consumption and production (SDG 12), resource efficiency and circular economy into conventional and holistic life cycle assessment to enable a fairer comparison of bio-based products and fossil-based products. The proposed hybridised cross-functional indicators were developed combining life cycle indicators with that of industrially-applied green chemistry resource efficiency metrics, also incorporating the principles of circular economy. The effectiveness of these methodologies was assessed via an exemplary comparative LCA of bio-based PLA packaging films with a fossil-based commercial equivalent from manufacturing to end of-life phases. In addition to the conventional impact indicators including land-use, freshwater and terrestrial eutrophication, water scarcity, fossil-resource use, acidification, quantitative and qualitative hybridised indicators such as hazardous chemical use, waste factor, secondary resource efficiency, energy efficiency and circularity have also been applied. The methodologies provided a unique dimension to the sustainability evaluation of bio-based products in terms of highlighting some of the "easy to interpret" impacts, particularly waste generation, material and energy efficiency of the product and process in question.

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How to simplify the assessment of life cycle impacts for new organic reactions? Development of a tool and application to a catalytic reaction.

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Growing concerns regarding the diminishing of feedstocks and energetic resources and increasing environmental pollution problems have led the chemical community to redefine its way of thinking to an approach to a vision directed towards sustainable development. In this context, modern chemistry is developing around the concept of "Green Chemistry". Since 90's, simplified metrics such as the E-factor is used. Such metrics are limited because they cannot assess in a holistic way all the environmental impacts arising from the life cycle of the products. Nevertheless, application of Life Cycle Assessment to new developments at the lab scale is not trivial because it requires expertise and data that are not necessarily known by the chemist. Also it is complex to model the impacts of new products at industrial scale. To tackle these issues, we developed a methodology and an associated tool that enable to assess environmental impacts of new chemical products with data that are easily accessible for the chemist at the lab scale. From these data, quantity of inputs, generation of waste, and energy use during the reaction at both the lab and industrial scale are quantified. This is done with a set of assumptions and modelling approaches about the nature of chemicals and energies, the question of recovery and treatment of them by distillation, incineration and waste water treatment adapted for the fine chemical industry. It results in precise life cycle inventory foreground data that can be included in SimaPro software using ecoinvent database for background data to compute life cycle impacts. This procedure has been tested for comparing different catalytic systems for the aldol reaction metal catalyst, organo-catalyst, and enzyme. Input data at the lab scale were collected from the chemistry literature. It shows that for all reactions, solvent production as well as waste treatment (through distillation/incineration or waste water treatment) has an important contribution to impacts. Modelled impacts at the industrial scale are therefore sensitive to the quantity of solvents used and to the fate of the waste and co-products. It is also possible to compare the catalytic reactions at both the lab scale and at the industrial scale considering different assumptions regarding quantity of solvents used, recovery rates by distillation, etc. This study shows that LCA can inform chemists on the greenness of their catalytic systems with quantitative values.

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An integrated Life Cycle Assessment and Risk Assessment approach for assessing the environmental sustainability of nanoproducts

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In the context of the recently finalized H2020 project, NanoReg^2, a new framework for a more comprehensive assessment of the environmental and human implication for engineered nanomaterials (ENMs) and related nano-enabled products has been developed. The developed framework is based on the integration of Life Cycle Assessment (LCA) and Risk Assessment (RA) methodologies during each stage of the innovation process for ENM products. Following a standard stage model, the framework is structured into four distinct phases: in stage one ("business idea"), the core business idea is described, an initial qualitative scoping of possible potential impacts and risks is established and the main legislative requirements are considered; in stage two ("business concept"), a simplified screening LCA-RA of potential benefits and risks (for workers, consumers and the environment) is performed, allowing a first rough evaluation of the sustainability of the product, based on qualitative/quantitative data from literature; stage three commences as experimental testing starts (stages 3a and 3b-"Prototype ENM/Prototype industrial sector"), and entails the undertaking of an LCA and RA at the lab and industrial scales, providing a first quantitative evaluation of the product; in the fourth and final stage ("validation and market"), REACH, product-related regulations/requirements and labelling requirements are integrated into the assessment. At each stage, different tools (e.g. LICARA, NanoRiskCat, Nanosafer, Swiss precautionary matrix) are used to provide valuable insights into performance to support the company in the process of the evaluation of the risk and sustainability of their product. Thus, our framework serves to help identify and minimise risks at an early stage in the innovation cycle and ensure that safety and sustainability implications are considered from the onset. For emerging products this is of high relevance as it enables risk and product management options to be enacted prior to the nanoproducts entering the market. In this presentation, this stage-based, integrated RA-LCA framework will be presented in more detail and its applicability will be demonstrated through several case studies regarding ENM production and application. In addition to the LCA and RA activities, the feasibility for adding a socio-economic component to this framework will be discussed.

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How can process simulation be used to get more reliable data for LCA of mining emerging technologies?

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In the context of the Raw material initiative launched by the European Commission in 2008, the European funded project IMP@CT aims at unlocking the access to small complex mineral deposits existing in Europe. To unlock this access, the project's goal is to develop modularized mobile plants (MMP) that can economically operate different type of ores in different types of geological and geographical contexts. More specifically, the project aims at deploying a pilot MMP that contains new technology methods related to mining, mineral beneficiation and waste and water management. The environmental impacts of the developed new technologies is assessed through Life cycle assessment (LCA), notably the potential benefits against business as usual (BAU) solutions. Two challenges arise in this environmental assessment: 1/ the upscaling effect: how can we compare pilot technologies to BAU ones? 2/ contextual assessment of IMP@CT technologies: how can we provide conclusions depending on the geological and geographical contexts? The first challenge is met using mineral process simulation, which is used to predict plant performances based on experimental data. The simulator is built on experimental data using USIM PAC^{TM} and consists in a complete flowsheet design along with a material model definition and several unit operations models adjusted on experimental mass balance. Outputs of the simulator consist, for each unit process of the plant, in flow rates (solids and liquids), consumption of energy and reagents for example. The second challenge of adaptability is met by creating an Excel tool that links the results of the process simulation with additional models, based on expert's judgments and literature data, to complete the whole foreground modelling and with information on the goal and scope that are based on the geological and geographical contexts. This tool's outputs are the foreground modelling of one ton of operated ore using IMP@CT developed technologies. This modeling is translated into ecoinvent 3.4 to be imported and assessed through Simapro 8.5 software. To use mineral processing alongside LCA allows the environmental assessment of emerging technologies to be built on more reliable data notably when upscaling effects arise. The building of the specific tool gives the adaptability to assess different kind of scenarios. At the end, results obtained can be used by decision makers for both eco-design approaches and benchmark assessments.

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Poster spotlight

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Wastewater Effluents: How Research Can Improve Risk Assessment and Regulation

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Targeting bioactive compounds with a combination of specific in vitro screens and high-performance thin-layer chromatography
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Micro-pollutants (MPs) exhibiting (eco)toxicological effects pose a well-known threat for environmental and human health, if released into the environment e.g. by wastewater treatment plants (WWTPs). The assessment of wastewater quality based only on indicator compounds does neither provide a reasonable evaluation of possible wastewater-associated adverse effects nor the possible generation of bioactive transformation products (TPs) by the treatment. *In vitro* bioreporters allow the analysis of the biological activity of a sample; however, the detected activity may not be directly linked to the identification of the substances that are responsible for the observed effect. The detection of bioactive compounds within an effect-directed approach (EDA) can be achieved by combining high-performance thin-layer chromatography (HPTLC) with bioreporters, which enable the assessment of the bioactive composition of the sample by a generation of activity profiles even in complex matrices. Furthermore, these activity profiles allow to determine a compound-specific effect-based elimination efficiency of WWTPs and thus the comparative assessment of various treatment technologies. Based on previous findings demonstrating the combination of HPTLC with the yeast estrogen screen (YES), we developed a comparable approach for genotoxicity (recA), androgenicity (YAS), thyroidogenicity (TR), dioxin-like effects (YDS) and effects on the vitamin D receptor (VDR). The already developed setup contains: (i) extraction and enrichment of MPs and possibly their TPs by solid phase extraction from environmental samples, (ii) separation of extracts and model compound mixtures using HPTLC, (iii) adverse effect quantification of the individual separated compounds using genetically engineered yeast (*Saccharomyces cerevisiae*) or bacterial (*E. coli*) bioreporters. The physical removal of separated compounds from the HPTLC plates and their identification using HPLC-MS is currently under development. Within our study we demonstrate the successful performance of the bioassays recA, YAS, TR, YDS and VDR on HPTLC-plates using several model compounds. The applicability of the developed methodology was demonstrated by the analysis of enriched influent and effluent extracts from a WWTP. We anticipate our methodology to substantially broaden the spectrum of specific endpoints combined with HPTLC for a more sophisticated screening of environmental samples such as sediment extracts, surface- and wastewater.

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The chain approach on pharmaceuticals in the Netherlands: a wicked problem.
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Wastewater Treatment Plant effluent with pharmaceutical residues is discharged into surface waters, which is raising substantial societal concerns. Because of these concerns, the Dutch government, together with many stakeholders from the health and water sectors, has developed a so-called chain approach to reduce the emission of pharmaceuticals to surface waters. This issue meets all the characteristics of a wicked problem, as it is a complex problem that is characterised by scientific uncertainties, multiple stakeholders with different values and interests, institutional complexity and lacks an easy solution. Within this chain approach, source measures as well as end-of-pipe measures are identified and, where feasible and effective, implemented. In this presentation, the development of the chain approach and a number of possible measures are discussed. Some measures are relatively easy to implement, such as as a campaign to reduce the amount of fluid leftovers to be disposed of in the sink. However, many measures seem relatively easy to implement but in practice appear to be a wicked problem in itself. Some examples are discussed, such as collection of X-ray contrast media using urine bags, collection of left-over pharmaceuticals at the pharmacy, source measures to control emissions of cytostatics, identification of pharmaceuticals to replace harmful pharmaceuticals, upgrading sewage treatment plants. The feasibility of these measures and the problems faced when implementing these measures is discussed.

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Sulfamethoxazole in the Vecht catchment: An application of the GREAT-ER model to assess antibiotic concentrations in whole river catchments
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Geo-referenced simulation models can support chemical exposure assessment in large river systems. Besides identification of hotspots, such mechanistic models are applied to gain a better understanding of the fate of chemicals in the environment. The geo-referenced exposure assessment tool for European rivers (GREAT-ER) has been designed to predict spatially explicit concentrations of 'down-the-drain'-chemicals in rivers. In this study, the model is used to predict the fate of the antibiotic sulfamethoxazole (SMX) in the catchment of the river Vecht (Germany, Netherlands). SMX has been identified as one of the antibiotics of particular concern in aquatic environments. Predicted environmental concentrations of SMX are of the same order of magnitude as those from monitoring campaigns carried out in European rivers. Sewage treatment plant (STP) Nordhorn is identified as one of the main emission sources of the river Vecht. A scenario in which this STP is theoretically equipped with a tertiary treatment stage showed a decrease of maximum average concentrations by a factor of 3. The probability of exceeding an ecotoxicological PNEC of 0.59 μg/L is relatively low in the Vecht catchment. Furtheremore, the PNEC for resistance selection (16 μg/L) is far above the maximum of simulated SMX concentrations (0.81 μg/L) in the Vecht catchment. Therefore, no actual risk for the aquatic environment is predicted from SMX emissions via household wastewater in the Vecht catchment.

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Toxicity removal by advanced wastewater treatment: A systematic review of effect-based studies
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The discharge of conventionally treated wastewater represents a major point source of micropollutants. To address this issue, several countries either consider or have already started to upgrade their wastewater treatment plants (WWTPs) with technical solutions such as ozonation or activated carbon treatment. An evaluation of these technologies on a per-chemical basis covers only a small fraction of known micropollutants and may, thus, not represent the actual removal performance. In addition, gaps in knowledge regarding the occurrence of unknown compounds, transformations products, and potential mixture effect exist. To overcome these limitations, effect-based measurements are increasingly integrated into the evaluation of advanced wastewater treatment plants, given that bioassays provide a more comprehensive picture of their performance in removing toxicity rather than single chemicals. However, while several reviews of target micropollutants exist, a systematic review of effect-based measurement data is lacking. Within the framework of a systematic review, we searched Web of Science for studies investigating pilot or full-scale ozonation or activated carbon treatment by effect-based methods. This search returned 2464 studies, from which we removed studies published before 2000, reviews and duplicates. Subsequently, we screened 2101 abstracts and 136 full-texts using pre-defined exclusion criteria resulting in a final set of 45 studies. We extracted the data from 47 *in vitro* bioassays and 17 *in vivo* model organisms and performed a quantitative evaluation of *in vitro* data by calculating removal rates for the different treatment options as well as a qualitative evaluation of *in vivo* data according to the reported adverse effects. Based on this extensive data set, we will give an overview of applied methods, summarize consistent and contradictory results, and highlight identified research gaps. Moreover, to critically assess whether upgrading WWTPs is beneficial, we will discuss the collected data set in the context of recently proposed effect-based trigger values.

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Ozonation alters toxicity of pharmaceuticals
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University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; G. Carlsson, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; O. Golovko, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; L. Norrgren, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health; J. Weiss, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; S. Örn, Swedish University of Agricultural Sciences / Department of Biomedical Sciences and Veterinary Public Health
Pharmaceutical residues are polluting the surface water environments worldwide. Sewage and wastewater treatment therefore needs to be improved in order to remove pharmaceutical residues from the effluent. One such treatment improvement is effluent ozonation. Even though ozonation has proven to be very efficient in reducing pharmaceutical parent compound concentrations in wastewater effluents, much remains unclear regarding potentially toxic ozonation by-product (OBP) formation. In this study we sought to elucidate toxic response of ozonated pharmaceuticals in zebrafish (*Danio rerio*) embryos in a fish embryo toxicity (ZFET) assay. Three pharmaceuticals commonly detected in wastewater effluents were evaluated for embryotoxicity before and after ozonation, i.e. carbamazepine, diclofenac, and oxazepam. Chemical analysis showed that carbamazepine and diclofenac were largely removed by ozone (90±11% and 97±3.8%), whereas oxazepam was removed to a lesser extent (19±5.7%). However, the ZFET assay revealed diverging toxicities. Diclofenac toxicity decreased with increasing ozonation. Oxazepam did not cause toxicity in the ZFET assay either pre- or post ozonation. However, indications of altered larval behavior in 6-day-old larvae were detected in the ozonated oxazepam treatment group. Carbamazepine toxicity on the other hand, increased with increasing ozonation. Chemical analysis showed the formation of two OBPs (carbamazepine-10,11-epoxide and 10,11-dihydrocarbamazepine), potentially explaining the increased toxicity. In conclusion, we have shown that while diclofenac toxicity was effectively removed by ozonation, carbamazepine embryotoxicity increased, possibly via formation of OBPs. The results of this study highlight the importance of new chemical and toxicological knowledge regarding the formation of OBPs in post-ozonated effluents. Work to establish OBP identities and toxicities (individually and in mixture) is ongoing.

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Emerging Contaminants in Water Systems: Potential Health Impact of Wastewater Reuse as Management Strategy for Water Security in South Africa

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South Africa as a country is water stressed but the growing effects of climate change with its extreme weather conditions and erratic rainfall pattern is aggravating the situation with incidence of droughts in many parts of South Africa, and Cape Town in particular, which is a major tourist centre in South Africa, the city experienced its major drought recently with attendant water scarcity scenarios in the city with the possibility of "Day zero" has been stated by provincial government when taps may run dry in the city. As a result of the impact of drought, water security will be a concern for many years to come in South Africa with consequents effects in people's health and economic activity in the city. One of the water management strategies being effected by government is promotion of wastewater reuse both domestic and agricultural use. However with the increasing concern on the occurrence of some pollutants of emerging concern in the water and wastewater systems in South Africa like the endocrine disrupting chemicals, there are concerns that these chemicals can pose a health threat to people especially as current technologies being used by many municipalities for water and wastewater treatment are not equipped to remove these chemicals. Many EDCs of are persistent in the environment and have been associated with several disease in humans. Our studies in the Cape Town environment have indicate the presence of such chemicals like BRFs, PFOS and pharmaceutical residues and for instance our health risk bioassay gave indication of slight risk of developing cancer through accidental ingestion via recreational activities . Ames tests result revealed clear mutagenic activities from wastewaters from livestock agricultural farms. High levels oestrogenic activity were also found in some of the wastewaters. If the waters were used for domestic purposes and sufficient treatment are not made, or if the water was used for irrigation purposes, greater risk would be expected. Our study also proposed a wastewater treatment technology using β-FeOOH/NiO nano composite material to remove EDCs from the wastewaters before reuse. *Table 1 could look like this*

Organic Micropollutants in Urban Waters (I)

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Temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers

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Several studies constrain a wide distribution of pharmaceuticals in rivers and streams. However, little is known about partitioning of pharmaceuticals between the water phase and suspended particulate matter (SPM). To close this gap of knowledge, we developed and validated a sensitive and high throughput analytical method for the analysis of 56 pharmaceuticals, 45 metabolites and transformation products (TP) as well as the artificial sweetener acesulfame sorbed to SPM. The method was based on pressurized liquid extraction (PLE) followed by a clean-up via solvent exchange and detection via direct injection-RPLC-MS/MS and freeze-drying-HILIC-MS/MS. SPM of annual composite samples were analyzed to determine the pharmaceutical concentrations between 2005 and 2015 at four sites of the river Rhine: Weil, Iffezheim, Koblenz and Bimmen as well as between 2006 and 2015 at one site of the river Saar, at Rehlingen. In these SPM samples, up to 61 of the 102 pharmaceuticals were detected with concentrations ranging up to 189 ng/g for guanyl urea, a transformation product of the antidiabetic metformin. The annual concentrations exhibited for most pharmaceuticals a good correlation with the consumption quantities in Germany. Partition coefficients (Kd) were determined for 91 analytes. They ranged from 0.64 L/kg to 9300 L/kg. For 19 pharmaceuticals Kd values were even found to be above 100 L/kg. Comparison of measured concentrations of pharmaceuticals in water with those measured in SPM showed that experimental partition coefficients can be used to estimate the water concentrations based on SPM concentrations. Benefits and the scope of SPM analysis for environmental monitoring of pharmaceuticals are discussed. Especially the possibility of the analysis of pharmaceuticals having elevated Kd values to calculate corresponding water concentrations is confirmed.

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Does the use of organic substrates favour the degradation of Emerging Organic Contaminants during artificial recharge of aquifers?

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The reuse of impaired waters has become a required component of water resources management due to the increasing demand of quality water, especially in arid and semiarid regions. Therefore, the development of sustainable, high-efficiency, low cost technologies for water treatment is urgent. Managed artificial recharge of aquifers (MAR) through infiltration basins is a low-energy and low-cost water recycling technology which allows to improve the recharge water quality and to increase groundwater resources. Wastewater treatment plant's effluents are very often used as a water source for MAR through infiltration basins. Emerging organic contaminats (EOCs) are not completelly removed during conventional treatments. Therefore, to understand the fate and transport of such contaminants during MAR is essential to select the possible location, to design the MAR system and to avoid aquifer contamination. The objective of this study is to design and test reactive barriers based on organic substrates to prevent leaching of EOCs, pathogens, and nutrients by increasing sorption surfaces and by releasing dissolved organic carbon to the infiltrated water, promoting diverse redox conditions and microbial communities. Six meso-scale system of artificial recharge were built inside a wastewater treatment plant facilities in order to compare the efectiveness of the reactive barriers and the role of diverse experimental conditions. Each one of the six systems (2.5 x 15 m) emulate an aquifer with an infiltration basin. The recharge areas of five systems were complemented with reactive barriers based on organic substrate. The system containing only sand in the recharge areas was stablished as the reference system. The dissolved organic matter (DOM) released by the reactive barrier into the infiltrated water was enough to achieve a broad range of redox conditions. Preliminary results from passive samplers show a better performance of the systems operating with the reactive barrier.

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Sources and environmental levels of pharmaceuticals in the Baltic Sea region

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The active pharmaceutical ingredients (APIs) have beneficial effects on human and animal health, but their undesired occurrence and effects in the environment is a global concern. The EU Water Framework Directive (WFD) considers the contamination of water with API residues as an emerging environmental concern. Baltic Sea has a large catchment area inhabiting 85 million people. Residues of e.g. hormones, anti-inflammatory and analgesics and antibiotics have been detected in the BS water or fish, but their environmental fate and effects on biota are still poorly known. EU WFD obliges member states to monitor certain APIs which may get legally binding environmental quality standards in surface waters

and emission reduction measures in the near future. HELCOM's status report on pharmaceuticals in the Baltic Sea region (BSR) provides a preliminary assessment that serves as a background for regional environmental policy regarding pharmaceuticals. The overall aim of this study is to fill in data gaps, identified by e.g. HELCOM, in order toget a more complete picture of significant sources and the extent of contamination by pharmaceuticals within the BSR. This information will in turn be used to evaluate environmental risks and to recommend efficient measures to reduce pharmaceuticals entering the Baltic Sea. Sampling has been performed in selected river basin districts of Finland, Estonia, Latvia, Poland, Germany and Sweden. Data collection covered emissions from point sources such as municipal wastewater treatment plants (MWWTPs), manufacturing facilities, hospitals, fish and livestock farms, as well as input of pharmaceuticals via agriculture, aquaculture and landfills Total of 55-75 APIs were analysed from WWTP influents and effluents, surface waters and sea water. Sample analysis is still ongoing, but first results show that that main source of API in the environment is unsufficiently treated WW from WWTP. The highest concentrations of APIs were found representing groups of anti-inflammatory drugs and drugs connected to cardio vascular deseases. API results of surface water showed huge variation of API concentrations between different countries and sampling points. The most environmental risky compounds will be pointed out for further necessary regulatory and emission reduction actions. Research was performed in the framework of EU Interreg BSR project "Clear waters from pharmaceuticals – CWPharma" (R055).

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Assessment of input of organic micropollutants and microplastics into the Baltic Sea by urban waters
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Urban waters, i.e., treated and untreated wastewater, treated and untreated stormwater as well as combined sewer overflows, are known emission pathways for micropollutants and microplastics into marine waters. Based on a model city with an exemplary sewer system and measured concentrations in the single sources, the relevance of the different emission pathways were modeled for different micropollutants. Additionally, the urban water catchment of the Baltic Sea and the associated water flows via the different sources were estimated. Subsequently, the emissions of micropollutants from urban areas to the Baltic Sea were assessed. According to our assessment, most micropollutants reach the receiving waters via treated wastewater (e.g., diclofenac). Thus, only for compounds with very high removal rates during conventional wastewater treatment, combined sewer overflows and untreated wastewater (due to misconnections in separated sewer systems or houses not connected to the sewer systems) become the dominating pathways (e.g., the disinfectant triclosan). Additionally, those compounds being present predominantly or solely in urban runoff (e.g., film-preserving biocides like terbutryn), stormwater and combined sewer overflows are the predominant pathways. As both, wastewater and stormwater treatment, are removing microplastics from the water phase to a high extend, untreated wastewater, untreated stormwater and combined sewer overflow are the predominant sources for input of microplastics into the Baltic Sea. Based on the measured concentrations in the different sources and the estimated water flows, the total mass loads of the single compounds entering the Baltic Sea reached up to several tons per year.

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Identification and removal of hazardous substances in Estonian municipal wastewater treatment plants
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Identification and removal of hazardous substances in Estonian municipal wastewater treatment plants Chemical pollution poses a threat to the aquatic environment and to the human health. Wastewater treatment plants are the first defensive line between aquatic environment and emissions of pollutants. This study focuses on identification of most relevant priority substances in Estonian municipal wastewater and their removal rate in treatment process. In total, 72 spot samples and 48 composite samples among with 32 sewage sludge samples were collected from influents and effluents of nine MWWTs and analyzed for 282 different substances. Potential sources for hazardous substances were identified using substance flow analysis methodology. Wastewater analysis showed that 143 substances were not found above LOQ. Di-2-ethylhexylphthalate (DEHP), toluene and heavy metals (As, Ba, Ni, Pb, Zn) were found from all samples. For some of the substances seasonal (e.g. diuron, glyphosate, trichloromethane) or population density (e.g. tetrachloroethene was detected only from MWWTPs with capacity more than 10 000 PEs or median concentrations of trichloromethane increased linearly according to the plant size) patterns could be detected. Yearly loadings of hazardous substances into MWWTPs as well as possible emissions to the environment were calculated based on actual measurements and flow rates of selected wastewater treatment plants. Table 1 shows loadings of relevant hazardous substances in Estonian MWWTPs. Substance Influent Effluent Sewage sludge kg/y kg/y kg/y Zinc (Zn) 4 923 1 533 14 888 AMPA 27 48 12 Di-2-ethylhexylphthalate (DEHP) 133 42 302 Glyphosate 16 26 6 Lead (Pb) 406 18 388 Trichloromethane (chloroform) 31 15 4 4-Nonylphenol 5 0 26 Toluene 169 8 27 Anthracene 1,3 0,1 1,5 Diuron 1,5 1,9 0,1 Table 1. Loadings of relevant hazardous substances in Estonian MWWTPs in 2017-2018 (kg/year) This study showed that many substances that are subject to international restrictions (e.g. Stockholm Convention, REACH) are still present in raw sewage and treated effluent. For most of the substances present in influent removal in MWWTPs was observed, but the chemical analyses of sewage sludge indicate that pollutants are often transmitted from water to the biomass.Table 1. Loadings of relevant hazardous substances in Estonian MWWTPs in 2017-2018 (kg/year)

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Mining the chemical information of urban wastewater - Monitoring human exposure to phosphorous flame retardants and plasticizers (PFRs)
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Phosphorous flame retardants and plasticizers (PFRs) have been increasingly used in the past years. Because of their ubiquity and the multiple exposure routes, they may pose a threat to human health. At the individual level, exposure to contaminants is generally assessed through the analysis of specific biomarkers in biological matrices. However, these studies are subject to limitations regarding monitoring of exposure in the general population over time and space. In particular, they are expensive, often lack a temporal dimension and suffer from selection bias. In this perspective, wastewater analysis complements human biomonitoring by gathering population-wide information about exposure to contaminants. This work focused on exploring the possibility of monitoring human exposure to PFRs through the analysis of urban wastewater. Solid-phase extraction and liquid chromatography tandem mass spectrometry were used to analyse selected biomarkers. Both spatial and temporal differences in levels of selected biomarkers were investigated by collecting samples in different locations and time periods. Substantial variability could be highlighted from a geographical perspective, suggesting that differences in exposure to PFRs exist among the investigated catchments. Time trends between 2013 and 2016 were investigated for one location and significant patterns could be highlighted. In general, these trends were in line with results from human biomonitoring studies and reports about production volumes. Finally, using stochastic urine production models and Monte Carlo simulations, attempts were made to estimate average urinary concentrations. Obtained concentrations were generally higher compared to results from human biomonitoring surveys. This could be linked to uncertainties in the calculation approach, input from external sources or differences in the sampling period (i.e., wastewater samples are collected over 24h, whilst spot urine samples are used in human studies).

Ecological Impact and Management of Dumped Munition Sites

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Sediment CWA and explosives hot spots in the Baltic Sea
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Chemical and conventional ammunition dumped in the Baltic Sea and the Skagerrak contain a wide range of hazardous substances. Considering the growing use of the seabed for economic purposes (offshore wind farms, pipelines, cables etc.), the likelihood of disturbing dumped containers with chemical warfare agents, or sunk mines and unexploded ordnance, causing direct emissions to the surrounding environment and risk of human and wildlife exposure, is increasing. Those munitions, while exposed to sea water, corrode and lose containment, leading to leakage and pollution of nearby sediments. Substances contained therein degrade in time, resulting in pollution of sediments with a mixture of parent compounds and their degradation products, which may be a point source of pollution for the ecosystem. Studies of sediments close to chemical and conventional munitions were performed in Bornholm, Gotland and Gdansk Deeps, as well as Kiel Bay. Pollution of sediments with degradation products of chemical warfare agents, explosives, and mercury coming from munition primers is observed within all studied dumpsites. The magnitude of associated concentrations is low, but spreading to larger area (up to 100m radius) was observed. Taking into account large number of munitions in the Baltic Sea bottom, such local sources may have a real impact on the environment.

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Tissue concentrations of chemical warfare agents and multi-biomarker responses in cod (Gadus morhua) from munition dumpsites in the Baltic Sea

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The main chemical warfare agent (CWA) dumping sites in the Baltic Sea are located east of Bornholm and southeast of Gotland. Based on official data, approximately 50 000 tonnes of chemical ammunitions were dumped in the Baltic Sea, mainly in the Bornholm Basin. Previous studies have shown that sediment samples collected from Bornholm area are contaminated by (CWAs) as a result of leaking from the dumped containers. These leaking toxic chemicals, e.g. arsenic-containing and mustard-related chemicals are posing a threat to the marine environment. Quantitative analysis of these chemicals and their degradation products are needed to prove the presence of these CWA-related chemicals in marine biota in order to support the risk assessment for possible accumulation of the substances in the food web. In order to study the uptake of arsenic-containing CWAs in fish in a larger scale, 100 cod (Gadus morhua) samples were collected from the Bornholm dumpsite and analysed for CWA-related phenylarsenic compounds. Also methods for the determination of biological responses (early warning biomarkers) and fish health status were applied in order to study their linkages to the chemical concentrations. Additional 100 samples were collected from the Gdansk Deep reference area. Based on chemical analysis, 13% of the analysed cod muscle samples collected from the Bornholm dumpsite contained CWA-related phenylarsenic compounds at parts per billion (ppb, ng/g) concentration levels. Statistically significant differences were found in most of the enzymatic biomarkers between the dumpsite and the reference site. However, biomarker levels measured from the cod containing the CWA-related arsenic compounds did not differ significantly from the other, "clean" specimens collected at the dumping site. Fish health status will further be studied and correlation with chemical and biochemical response will be evaluated.

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When lead is dangerous in water environment? Factors influencing on lead transfer from spent gunshot to deposits and water.

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Lead fishing sinkers and spent gunshot are the main sources of lead poisoning among waterfowl. They are also suspected to directly pollute water and deposits, but the conditions under which they would have not been determined.I conducted anexperiment aimed at evaluating the impact of lead pellets in microcosms (n=160) built with two deposit types (mud and gravel), three water pH (4, 7 and 9) and two wind levels (wind and windless simulation). Strong differences in Pb transfer (measured with ICP-OES) between deposit types and pH of water were observed. Wind simulation was a significant factor but only in the case of gravel deposits. The strongest lead input to water and deposit occurred in mud microcosms with a water pH of 4. Median pellet loss during experiment did not differ much between deposit types. The experiment revealed that Pb from spent gunshot is only transferred to the environment in specific environmental conditions. This suggestion is crucial for planning the restoration policy.

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Concentrations of chemical warfare agents and effects on meiofauna in a Skagerrak dump site

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Large stockpiles of chemical munitions were dumped after the end of WWII in deep-water areas in the Baltic Sea and Skagerrak. Approximately 65 000 ton chemical warfare agents (CWA), where put in obsolete ships that were sunk between 1947-1948. A deep-water area used for dumping was the so-called Måseskär dumpsite in Skagerrak (27x 21 km), where only unconfirmed information exists of if and how much CWAs that was dumped. In a survey 2015 the area was thoroughly investigated in a hydrographic survey, where 28 wrecks were detected. An expedition to the Måseskär dumpsite area was performed between 17thand-22nd of June 2017. It is located approximately 14 nm west of the island of Måseskär, on the Swedish west coast. The depth in the area is between 190-220 m. Five areas were sampled for sediment using a Niemesto corer (Ø 7cm). Two of the areas were in the vicinity of the ship wrecks (0.5-5 km), one site in between to ship wrecks areas, one area north west of the area with the highest concentrations of wrecks (downstream, 24-29 km to the closest wreck) and the last area was a control area south of the dumping area (22-26 km from the closest ship wreck). At each area five sites were sampled, except for the area with highest concentration of wrecks (cluster 4), were 9 sites where sampled. Mustard gas or degradation products of mustard gas were detected in 13 (45%) of the sediment samples. Concentrations in those samples ranged from below limit of detection to 3.4 μg/kg dw (1,4-oxathiane), where the highest concentration were found in the area with highest concentrations of shipwrecks. Average As concentrations differed between 21.6- 8.5 μg/kg (dw) at 0-3 cm, 19.7-9.0 at 4-7 cm, 17.7- 8.1 at 8-10 cm and 19.2-7.4 at 11-15 cm, measured by XRF. Average As concentrations from 0-3 cm depth differed significantly between the sampled areas ($p < 0.001$). All of the sampled areas in the wreck area (C2-C4) had significantly higher concentrations of As compared to the reference area (C1). These areas had similar As concentrations and did not significantly differ from each other. Meiofaunal community composition differed significantly ($p < 0.01$) between the sampled areas. The reference area (C1) differed signficantly from the other sites, mostly due to a higher abundance of nematodes. In this study we confirm that the Måseskär area is a dumping area for chemical warfare agents, as degradation products of Mustard gas and elevated concentrations of As were detected in the sediment.

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Blue mussels (Mytilus spp.) transplanted at a German munition dumping site in the Baltic Sea for biomonitoring of TNT and degradation products

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Since World War I considerable amounts of warfare materials have been dumped at sea worldwide, but little is known about the fate of the explosive components in the marine environment. Sea dumped munitions are able to contaminate the surroundings because of the release of explosive chemicals due to corrosion and breaching or by detonation after blast-operations. This implies the risk of accumulation of toxic compounds in human and wildlife food chains. With the help of divers, we performed an active biomonitoring study with transplanted blue mussels (Mytilus spp.) in a burdened area (Kolberger Heide, Germany) with explosive compounds near blast craters. Furthermore, we transplanted mussels in vicinity to a mine mound with over 100 moored mines. In order to monitor any differences resulting from changing seasons, three exposure periods for mussels exposed at the mine mound were chosen. In all mussels deployed at the ground next to the bulk of hexanite, we found a body burden with 2-amino-4,6-dinitrotoluene (2-ADNT) of 103.75 ± 12.77 ng/g wet weight and with 4-amino-2,6-dinitrotoluene (4-ADNT) of 131.31 ± 9.53 ng/g wet weight. 2,4,6-trinitrotoluene (TNT) itself has been found in six mussels with an average concentration of 31.04 ± 3.26 ng/g mussel wet weight. In the mussels positioned at one meter above the ground no TNT nor 2-ADNT could be detected, but 4-ADNT was found in all samples with an average concentration of 8.71 ± 2.88 ng/g mussel wet weight. In mussel tissues from the first exposure period (April – July 2016, 106 days) 4-ADNT ranging from 3.37 ± 1.01 to 7.76 ± 6.53 ng/(g mussel wet weight) could be detected in all samples at each mooring position. After the second exposure period (July – December 2016, 146 days) 4-ADNT ranging from 3.04 ± 1.30 to 4.94 ± 1.60 ng/(g mussel wet weight) has been found in 34 out of 36 samples. After the third exposure period (December 2016 – March 2017, 92 days) 4-ADNT levels ranging from 4.26 ± 1.40 to 7.01 ± 2.41 ng/(g mussel wet weight) were detected in mussels from recovered positions. Neither TNT nor 2-ADNT have been found in any sample. With this biomonitoring system, we could show that blue mussels accumulate TNT and its metabolites 2-ADNT and 4-ADNT in their tissues and we unequivocally proved that toxic explosives accumulate in the marine biota resp. in the marine food chain, thereby posing a possible risk to the marine ecosphere and human health.

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Toxic effects of dissolved TNT on the Baltic mussels (Mytilus spp.): first results from lab exposure studies

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During and after the WWs, large amounts of chemical weapons and conventional munitions stored on German territory were dumped in the Baltic Sea by order of the allied forces or German administration. In addition of being a cheap method of disposal, the belief was that the vast amounts of waters in the oceans would neutralize and absorb the dangerous substances. Nowadays, dumped warfare agents are posing a growing concern for the marine environment since dangerous contents are leaking from corroding metal shells and pollute ambient sediments

and water. Both, chemical and conventional warfare agents and their degradation products have been detected in noticeable concentrations in sediments at the major dumping sites in the Baltic Sea. However, the knowledge about dissolved explosives on the health of marine organisms is scars. Even basic toxicity values such as lethal TNT concentrations are missing in literature for most organisms. Therefore, we conducted blue mussel exposure experiments in the lab to assess environmental impact of dissolved TNT. Biomarkers representing different biological functions including e.g. antioxidant defence, biotransformation, lysosomal membrane stability, cellular energy allocation and condition index were investigated. Moreover, tissue concentrations of TNT and its degradation products were analysed and correlated with the biological effects. First results revealed that mussels show clear reactions of avoidance and negative biological effects when they are exposed to dissolved TNT under lab conditions. For example, shell closure, a simple mechanism to avoid any unfavourable conditions, leads to long exposure times under high concentrations of toxic compounds until lethal doses is reached. In contrast, concentrations of dissolved TNT where no negative effects on mussel's health were detectable were comparably low. Thus clearly demonstrating the overall toxicity of TNT for marine organisms.

Statistical Science and Ecotoxicology: Bright Lines and Dark Alley Ways

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Statistical ecotoxicology: A crisis of confidence, or, why does it hurt when I P (<0.005)?

D. Fox, Environmetrics Australia; R. Smith, Hydrobiology Pty Ltd; W.G. Landis, Western Washington University / Institute of Environmental Toxicology

It has been said that the integration of statistical methods into ecotoxicological practice has occurred more by osmosis than by design. This has resulted in a hybrid discipline we call *statistical ecotoxicology* that has evolved in a serendipitous manner having no mandate, no clear direction, and an unknown trajectory. In our various roles and capacities we have campaigned for a tighter, more transparent and well-articulated coupling of *statistical science* and *ecotoxicology* to ensure practitioners use methods of data collection and analysis that are fit-for-purpose; robust; scientifically credible and, statistically defensible. Given the recent calls to ban the use of Null Hypothesis Significance Testing (NHST) and (separately) to lower the default 0.05 level of significance criterion to 0.005 it is both timely and pertinent to contemplate what the most recent P-value brouhaha means for ecotoxicology. We conclude that it is time that the existence and importance of *statistical ecotoxicology* as a sub-discipline within our society was formally recognised and given a voice to provide guidance, training and leadership on important statistical issues in ecotoxicology. The status quo fosters uncertainty about best (statistical) practice in ecotoxicology and provides no clear mechanism for issuing guidance.

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Describing exposure-response to meet the requirements of ecological risk assessment

W.G. Landis, Western Washington University / Institute of Environmental Toxicology; R. Smith, Hydrobiology Pty Ltd; D. Fox, Environmetrics Australia

The use of null hypothesis signifcance testing (NHST)is still the dominant method of reporting exposure-response experiments although it has long been discredited. One of the reasons is that the output of an current ecological risk assessment is a series of probability distributions that describe the multiple outcomes of the interactions among of multiple stressors with multiple endpoints often with correspondingly different management goals. In order to accomplish this task an accurate and complete and probabilistic description of the exposure-response relationships and the environmental factors that alter the interaction be available. NHST approaches do not meet these needs. Risk assessment is akin to the approach of S. Wright in determing the causes of the expression of phenotypes (genes and environment) as opposed to the use of hypothesis testing supported by Pearson, Fisher and their disciple[s.]. Significance in a risk assessment is related to but not the same as for a hypothesis test result. Significance in a hypothesis test is used to make a decision regarding whether to accept or not accept that the result is due to a specific hypothesis. In risk assessment and adaptive management, the output is a probability distribution is used to make a decision on the type of management options that are taken to achieve a specific result with an acceptable probability of success. Fundamental to achieving the kind of information necessary to describe the interactions for a risk assesssmsent is the mapping of the exposure-response relationship, especially where there are dramatic changes in the response and when acted upon by factors such as temperature, cofactors, and nutrients.The reporting of NOEC, LOEC and other values derived from the NHST approach are not informative in current risk assessment techniques. Such reporting is not probabilistic and does not reflect the uncertainties in the exposure-response or other types causal interactions. However, significance, as part of a decision-making process does have an important role to play, especially in the evaluation of management options following a risk assessment process. Significance means that the outcome was informative in making a decision regarding the use of a particular managment treatment in the context of the social, economic and engineering priorities.

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Promoting data literacy to improve statistics in ecotoxicology

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Ecotoxicology statistics are still dominated by comparison approaches, like *t*-test and ANOVA. Often *post-hoc* tests follow, to determine significant differences between arbitrary treatment groups. The toxicity proxies are typically NOECs and LOECs, which today are disputed. The use and interpretation of *P*-values in this context is seen as highly critical. Dose-response modelling provides a different way of analysing data; however, its algorithms do not evaluate the effects of stressors, but optimize *a priori* curve parameters to fit data points. This results in EC_X/LC_X values, but is still not an analysis of stressor effects in the strict sense. More advanced types of data analysis are needed, e.g., using open source statistical software R. But R is still not broadly used within ecotoxicology. This is due to traditional data analysis following "recipes", without the need for developing literacy for data and its handling, including statistics. Consequently, a "inside-the-box" thinking is widely abundant when developing hypotheses, designing experiments, and analysing data. In ecotoxicology, there has been a traditional focus on strong quality standards for laboratory work, such as the GLP framework. Unfortunately, the same thoroughness is not applied to data, and ecotoxicology needs good data practice (GDP) for handling, analysis (including statistics), and reporting of data. This will increase the current standards of documenting and reporting data analysis, and lower the threshold to approach different statistical methods. We propose the development of transparent ways for reporting ecotoxicology data analysis, data handling, and data visualization. R can aid this process, providing frameworks for documenting and reporting code. In this scenario, GDP will increase usefulness of studies, while minimising investment in time and resources. With big data and data science firmly established as buzz words, it is high time to rethink our data and statistics culture, beginning with small data. We need to sensitize, educate, and train both established scientists and new researchers in principles of modern data handling and statistics. This will ready ecotoxicology for the exciting data and statistics developments ahead of us.

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Recommended procedures for the interpretation of histopathology data from environmental toxicology studies, including a repudiation of the index system approach

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Histopathology is a key endpoint for many environmental toxicological bioassays, as it can provide information relevant to hazard identification, risk assessment, the elucidation of disease mechanisms, monitoring of contaminant remediation efforts, and the general health of animal test subjects. Additionally, morphologic tissue changes can serve as critical links between initiating molecular events and apical effects in adverse outcome pathways. Despite the long established use of this venerable endpoint as a research tool, published recommendations concerning the appropriate collection, interpretation, and reporting of histopathology data for hypothesis-based ecotoxicological challenge studies are not widely available. In particular, issues arise when attempts are made to convert descriptive microscopic findings and semi-quantitative ordinal categorizations of lesion severity to quantitative data. Increasingly evident in the literature are papers in which the histopathology data are reduced to numerical index values per organ type and test organism. Although seemingly scientific, this inherently biased approach relies on a variety of unsupported assumptions and statistically invalid mathematical calculations. More importantly, because they promote the recording of numerous unrelated finding types, many of which are incidental to the study outcome, index systems are highly prone to the generation of false positive and false negative results. One very real danger is that inaccurate conclusions resulting from such analyses will be used in characterizations of hazard or risk to establish public policy. This presentation will illustrate the shortcomings of index systems, and then describe an algorithmic, weight-of-evidence approach for the recording, interpretation, and statistical analysis of histopathology data from toxicological studies that utilize fish as test subjects.

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Analysis of Non-Target Terrestrial Plant Studies

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Two types of Non-target terrestrial plant (NTTP) studies are required for registration of crop protection chemicals. Unlike many aquatic studies, it is not uncommon to test 8 to 10 treatment groups (application rates) or even as many as 12 to 14, in addition to one or two negative controls, with 4 to 10 replicates per application rate. There are three primary response: shoot height, dry weight and survival. Requirements include NOEC determination and ECx estimation, x=50 in

Europe and x=25 in the US. There has been recent interest in the US of requiring estimation of ECx for much smaller values of x, such as x=10 or even x=5, at least for the growth responses. Issues of both statistical and regulatory importance for the survival response include how to account for (1) background (or control) mortality and (2) replicate variability, including treatment of the overdispersion that is sometimes observed. Issues of importance for the growth responses include how to (1) model hormesis or low-dose stimulation, which is not uncommon, (2) how to assess the quality of ECx estimates for all responses, and (3) to determine what size effects can be estimated reliably from the data resulting from these studies. Another issue is whether the experimental design should be changed, either to improve the quality of the statistical analysis or to reduce the cost of these studies without sacrificing the quality of statistical conclusions. These issues are explored through real data examples and extensive computer modeling. Some common errors are demonstrated that can seriously bias results. Simple, practical recommendations are made to eliminate these errors and improve the quality of results.

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The hidden cost of not considering experiment-wise error rates in effluent monitoring
R. Smith, Hydrobiology Pty Ltd; D. Fox, Environmetrics Australia; W.G. Landis, Western Washington University / Institute of Environmental Toxicology
In the analysis of ecotoxicological datasets for both multi-species testing and for contaminant monitoring we tend to ignore the overall experiment-wise error rates. This probably results from a combination of the relative youth of our field of science, a determination to find fundamental or simple answers to puzzling and complex issues and a desire to provide conservative assessments that satisfy the perceived requirements of practitioners and regulators. Nonetheless, it does affect the practice of ecotoxicology in real-world situations and influences real world costs of environmental management. In this paper we will examine the general lack of control of experiment-wise Type I error rates in multi-species and multi-sample assessments of toxicity, arguably the most common practical application of ecotoxicology in environmental assessment. It might be argued that the common practice of applying α=0.05 for each test is a conservative trade-off that increases the statistical power to detect toxicity by an increase in the Type I error rate, but actual measurement of statistical power or experiment-wiseType I error rates are rare in ecotoxicology, so the value of this trade off is rarely known. The net result is that environmental management targets are specified that are based on layers of conservatism, and that have overall error rates that could be measured and reported, but generally are not. How much this costs society and operators in terms of compliance with overly-conservative guidelines is, therefore, not known and incalculable. We believe that it is possible to better report and measure these hidden error rates, and that better insight into them may well provide for more cost-effective management. Perhaps more strongly, failure to consider and manage experiment-wise Type I error rates is a disservice to both operators and regulators alike, and is eschewed in other scientific disciplines

Human Health and Environmental Risk Assessment of Chemical Mixtures: Moving Towards the Non-toxic Environment (I)

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The scientific and regulatory assessment of mixture ecotoxicology: a critical reflection on known knowns, known unknowns and unknown unknowns
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Making use of the experiences gained from the regulation of individual chemicals, various tiered approaches for mixture risk assessment have been suggested during the last decade, published by various national and international regulatory authorities, industry organizations and academics. Most often they are based on a combination of whole-mixture testing and component-based approaches, the latter making use of the classical mixture toxicity concepts of Concentration Addition and Independent Action (Response Addition), which are more and more often embedded in modern ecotoxicological approaches such as read-across methods, species-sensitivity distributions (SSDs), high-throughput testing, TKTD models (e.g. Debtox) and adverse outcome pathways (AOPs). This presentation will present a brief overview of how classic and modern approaches are combined for evaluating the ecotoxicology of chemical mixtures. It will then discuss why the science of mixture ecotoxicology is still insufficiently considered in the practice of chemical risk assessment and management. The following issues will be analysed in detail: (1) the adequacy of the empirical database: how much do we actually know about the ecotoxicity of complex, environmentally realistic mixtures? Do the classic mixture concepts provide a sufficiently reliable basis for predictive mixture assessments? Where are the limits of our current understanding? (2) the complexity challenge: how can we simplify the overwhelming complexity of chemical mixtures to make their assessment manageable in practice? (3) bias and uncertainty: how much accuracy and precision can we expect to achieve with modern approaches, how much do we need for taking regulatory decisions? (4) socio-economic and political consequences: what would/could actually happen if mixture assessments would be systematically implemented on a broad scale? In the end, I argue that the mixture issue is an almost classical example of the "tragedy of the commons", a situation that is simply outside the framework of current (actor-oriented) chemical legislation. Its systematic consideration might need to start with a hard look at the fundamental assumptions that underpin our current frameworks for chemical assessment and management. This presentation is specifically intended as a critical reflection on the state of the art and a discussion starter on the ways forward. The author welcomes any and all feedback.

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Development of a Multi-Substance Potentially Affected Fraction Method for Assessing the Joint Toxicity of Twenty-two Pesticides with Ten Modes of Action
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The health and resilience of the Great Barrier Reef (GBR), the world's largest reef ecosystem, is in decline. To improve this situation the Australian and Queensland governments developed the Reef 2050 Long-Term Sustainability Plan (2050 Reef Plan) and the Reef 2050 Water Quality Improvement Plan (WQIP). This WQIP has land and catchment management targets and water quality targets. The latter focus on reducing the annual loads (mass) of fine solids and dissolved inorganic nitrogen and the toxicity of mixtures of pesticides. Specifically, the target for pesticides is for at least 99% of aquatic species to be protected from the combined effects of pesticides at the mouth of waterways that discharge to the GBR. This level of protection is consistent with that recommended to waters in national parks and marine reserves by the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. The multi-substance potentially affected fraction (ms-PAF) method was selected to monitor progress to the pesticide reduction target. This method was selected as it expresses the combined effect of pesticides in terms of the percent of species that would theoretically be affected, which is consistent with the Australian and New Zealand water quality guidelines. The pesticides included in the ms-PAF method were selected because they have been analysed for and are frequently detected in waterways that discharge to the GBR, and they have water quality guideline values (GVs). The 22 selected pesticides included: 2,4-D, ametryn, atrazine, diuron, chlorpyrifos, fipronil, fluoroxypyr, haloxyfop, hexazinone, imazapic, imidacloprid, isoxaflutole, MCPA, metolachlor, metribuzin, metsulfuron-methyl, pendimethalin, prometryn, simazine, tebuthiuron, terbuthylazine and triclopyr. Species sensitivity distributions were derived for each pesticide using fresh and marine species. The groupings of pesticides for msPAF was determined by a combination of literature determinations of the MoA, tests for parallelism of the SSDs, and goodness of fit of each pesticide to global SSDs for various combinations of the pesticides. The toxic effects of pesticides with parallel SSDs (within the same MoA) were estimated using the concentration addition model, while the toxic effects of pesticides with non-parallel SSDs were combined using the response addition model. The ms-PAF method will be used to estimate the combined toxicity of the 22 pesticides to thirty-seven sites during 2017 – 2018.

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Impactful mixture exposures in Europe's surface waters and prioritizations towards a non-toxic environment
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Based on analyses of mixture data in the EU-projects SOLUTIONS and MARS we can conclude that the non-toxic environment has not been materialized for a large fraction of European surface waters. To reach this conclusion, we collated data on chemical production, emissions, concentrations and expected impacts, and on ecological status. SOLUTIONS – a project considering the European use and impacts of chemicals in the context of the concepts of Good Chemical Status – provided the former data, and MARS – a project considering the ecological status of European water bodies in the context of multiple stressor impacts – provided the latter. Based on Europe-wide modelling, the analyses of the SOLUTIONS data suggests that mixture exposures exceed the threshold that could be used to define the non-toxic environmental. By aligning the predicted impact data to ecological status data based on monitoring, it was found that indeed mixtures are one of the factors likely shaping ecological communities. That is, higher mixture toxic pressures impose a limitation to maintaining or reaching good ecological status. The findings are discussed in view of further validation data, monitoring and classification methods of the Water Framework Directive, and especially prioritization of management to forward the non-toxic environment.

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Antibiotics and their mixtures in aquatic and terrestrial ecosystems: do we underestimate risks to environmental organisms?

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The increasing occurrence of antibiotics in the environment is highly discussed nowadays. Antibiotics used in human medicines are continuously released into surface waters via waste water treatment plants (WWTP), because of incomplete removal, but may also enter agricultural lands via sewage sludge. Antibiotics used in veterinary medicines enter the terrestrial environment via manure and may be transferred to surface waters. Furthermore, antibiotics reach groundwater ecosystems per bank filtration and transport through soil. Environmental risks are considered in the environmental risk assessments of human (HMP) and veterinary medicinal products (VMP) within the authorisation of new medicinal products in the EU. In these procedures the risk of each active substance is assessed for itself. However, environmental organisms may be exposed to several antibiotic substances with a similar mode of action in environmental media like surface water and soil. The intention of the presentation is to show that there are risks to ecosystems arising from the antibiotic substances itself and from their mixtures in addition to the risk of formation of resistant microorganisms. Risks of antibiotic mixtures in the terrestrial and aquatic environment are evaluated by using data of authorisation procedures and from research projects funded by the German Environment Agency (UBA). Recently, risks for plants were identified in authorisation procedures of some VMPs. Many antibiotics e. g. tetracycline, tiamulin, tylosin, trimethoprim are persistent in the terrestrial environment. So, the probability of accumulation in soil and hence the formation of mixtures of different antibiotic substances in soil is high. Furthermore, the most sensitive organisms were identified for the risk assessment from tests of the different taxonomic groups in surface water. Results from an UBA research project demonstrate that antibiotic mixtures have a high relevance in the aquatic environment. The provided overview shows that environmental ecosystems are exposed to mixtures of antibiotics and effects on specific organisms are probable in all environmental compartments. As the implementation of risk mitigation measures is limited, measures to reduce the usage of antibiotics and to improve cleaning technologies are very appreciated by the agency.

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Spatial aspects of the Baltic fish dioxin risk formation: Social-ecological system analysis

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Dioxins have been a nuisance to the Baltic Sea ecosystem for a couple of decades, and even though the concentrations in the environment and biota have decreased, they pose a multilevel social-ecological risk. Dioxins accumulate to fatty tissue, and hence enrich in the food chain ending up to humans who consume fish. We analyzed the dioxin risk in the Baltic Sea using social-ecological system (SES) approach to assess the management of the dioxin issue in spatial terms. We studied the dioxin flux in the Baltic Sea food webs focusing especially on Baltic herring and salmon which are important commercial species and have prominent role in food web dynamics. A systematic literature search was conducted to explore how different elements in indicators of 1) anthropogenic dioxin pollution, 2) ambient environmental conditions, 3) food webs, 4) dioxin concentrations in fish, and 5) human exposure from fish affect the Baltic Sea SES. From 47 articles, reports, and theses, highly heterogeneous data, varying from *In situ* monitoring data to experimental and modelled information, was extracted to create a total of 21 elements to describe the Baltic Sea condition. Arising from the areal differences in dioxin pollution and environmental conditions affecting the ecology and biology, these elements vary spatially, affecting how the final risk is formed in different basins. In the Northern Baltic, Gulf of Bothnia, where dioxin concentrations in herring are the highest, the exposure to a key risk group, women in fertile age, is lower than in the Baltic Proper. On the contrary, salmon PCDD/F concentration is the highest in the Gulf of Finland, while the exposure to the risk group is the highest in the Gulf of Bothnia. This study highlights the importance of integrative SES when evaluating the need for area-specific risk management strategies.

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Poster spotlight

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The Environment as a Reactor Determining Fate and Toxicity of Nanomaterials (III)

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The Influence Of Soil Properties On The Uptake Of Different Ag Nanoparticle Forms By Wheat.

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Silver nanoparticle (Ag NP) addition to terrestrial environments occur through releases from anthropogenic activities. It is expected that most particles will no longer be in their pristine forms when they reach the soil but will have been transformed by their environment (e.g. sulphidisation during waste water treatment). The extent of aging of the Ag NP forms once they are in the soil is influenced by the properties of the soil, and will greatly vary the form of Ag NP to which soil organisms are exposed and the ultimate availability of the silver. The bioavailability of silver forms in amended soils is a key aspect to understanding the environmental risks associated with them. In order to develop models that support site-specific risk assessments we need a better understanding of how Ag NP transformation processes, fate and bioavailability are influenced by soil properties. The aim of this study is to investigate the uptake of silver by wheat *Triticum aestivum,* when exposed to three Ag forms (20nm Ag NP, 27nm Ag_2S NP and $AgNO_3$) in three natural soils with varying pH (4.7-6.5) and organic matter content (0.6-9.6%). Soils were spiked at 10mg Ag/kg and equilibrated for 7 days before seed planting. Exposures were conducted in triplicate and sampled at 1, 4, 6, 13 and 27 days post-emergence (PM). Soils were maintained at 60% water holding capacity and exposures conducted at 20 C with a 16L: 8D photoperiod. Soil pore water was extracted at 6 and 27 days PM (0.45μm and 10 kDa filtered) as a measure of Ag bioavailability in the soils. Roots washed in dH_2O were dried at 70 C and plant growth rates calculated on dry weights.Total silver concentration in the roots, shoots, soils and pore waters were measured by flame and graphite furnace AAS. This study not only shows that different Ag forms are differentially bioavailable to wheat in soil exposures but also that soil properties have a pronounced influence on their bioavailability. This study shows that environmentally relevant forms of Ag NPs (Ag2S) are bioavailable to wheat in soil exposures and that in the Woburn soil the root concentrations of silver from the Ag_2S NP exposure were even higher than those found for the pristine Ag NP or ionic Ag exposures in either Lufa or North Wales. The differences in root silver concentrations between soils seen in this experiment highlight the importance of understanding the environment in which Ag NPs are applied to predict the bioavailability of silver to soil organisms.

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Ecotoxicology of silver species brought by sewage sludge in terrestrial environment

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The rise of nanotechnologies in the 90s led to a larger use of silver nanoparticles (AgNPs) in many products, mainly due to their antimicrobial properties. Consequently, a discharge of silver (Ag) into wastewater leading to an accumulation of silver species in sewage sludge has been observed. In this context, a primary source of Ag contamination of terrestrial environments is related to sewage sludge land application for agricultural or remediation purposes. In terrestrial ecosystems, exposure to AgNPs (or Ag species contained in sludge) may change microbial biomass and diversity, decrease plant growth and inhibit soil fauna reproduction. Physiological, biochemical and molecular effects have been evidenced in various soil organisms and microorganisms. AgNPs undergo speciation before spreading on agricultural parcels. The effects of the dominant form in sewage sludge, Ag sulfide, are still too little studied. An experiment have been conducted to evaluate the impact of Ag species on soil microflora and on a macroinvertebrate. Objective was to assess the toxicity of Ag brought into the environment via sewage sludge spreading. The exposure was as realistic as possible. Briefly, earthworms were exposed to mixtures of natural soil and fermented sludge contaminated with Ag (contamination before anaerobic fermentation). Life traits and bioaccumulation of metals have been evaluated for earthworms, diversity and activities were evaluated for microorganisms. First results confirmed that the totality of AgNPs added in microcosms were transformed in sludge. These Ag forms impacted moderately the earthworm test species *Eisenia fetida*. Life traits were not affected by Ag although Ag accumulated in their organism. Gene expression levels of *lysenin*, *superoxide dismutase* and *Cd metallothionein* genes (involved in defense mechanisms against metals) were not affected by Ag in these conditions. The study of soil microorganisms shows that respiration, denitrification and nitrification were not affected by added of sludge contaminated by AgNPs. However, silver had a significant effect on the diversity of communities. Our experiment suggest that although Ag contaminated sludge does not appear to be extremely toxic to earthworms, Ag forms accumulated in animals are thus introduced in trophic chain. Also, the alteration of the diversity of soil microflora could affect long term soil fertility since communities of soil microorganisms are essential for soil fertility.

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Time-resolved leaching of engineered nanoparticles from sewage sludge ash

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Engineered nanoparticles (NP) are efficiently retained in wastewater treatment plants (WWTP) and accumulate in the sewage sludge. The application of sewage sludge as fertilizer in agriculture becomes increasingly difficult due to more stringent environmental regulations. Therefore, increasing shares of sewage sludge are incinerated, preferably in mono-combustion incinerators. To assess the potential release of NP from produced sewage sludge ash, we spiked Au, Ag and CeO_2-NP, representing NP of different reactivity, to a pilot WWTP consisting of conventional activated sludge treatment and anaerobic digestion. Digested sludge was collected, dewatered, dried and incinerated in a pilot fluidized bed reactor. Fly ash was packed into liquid chromatography glass columns and leached using a 53 mM NaCl solution. Individual fractions of the leachate were collected over time. Initial results showed that of the total mass of Au, Ag and Ce in the column, 3.3%, 0.1% and 0.003% was eluted during the experiment, respectively. Al and Fe release was assumed to represent colloid leaching from the ash. The concentration decrease of Au in the leachates followed a first order release and Au concentrations were not correlated with the leaching of Al and Fe. Ag release seemed to partially depend on the Al and Fe release. Ce release only occurred during intense Al and Fe release. Based on the target element release being positively correlated or independent of Al and Fe release, we assumed that Au was only loosely attached to ash matrix. Ag and Ce were partially and mostly incorporated into the ash matrix. Based on these initial results, we conclude that the type of chemical transformation affects the interaction between the NP and the sludge / ash matrix. These interactions seem to dominate the release of NP from sewage sludge ash. NP which are not expected to undergo major chemical transformation during sludge incineration (e.g., AuNP) will only be loosely attached to the ash matrix and thus easily elute. NP that undergo significant transformation and interact with the matrix during incineration (e.g. Ag or CeO_2-NP) will be retained in the ash to a larger extent.

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Transformation of cerium dioxide nanoparticles during sewage sludge incineration

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Wastewater treatment plants (WWTP) show high removal efficiencies for (engineered) nanomaterials, resulting in their accumulation in sewage sludge. An increasing percentage of the sewage sludge is incinerated in many countries, including Germany (~30%) and Switzerland (~70%). Given the predicted increase of cerium dioxide nanoparticle (CeO_2 NP) production and the related release into WWTP as well as the growing importance of sewage sludge incineration, knowledge on the physical-chemical transformations of CeO_2 NP during wastewater treatment and sludge incineration is required for a detailed understanding the fate of CeO_2 NP and assessing the (eco-) toxicological effects related to their increased use. Previous studies indicated that the complexity of the experimental system (e.g. type of incineration, matrix in which the CeO_2 NP are dispersed) influences the chemical speciation of Ce in the ashes. We therefore incinerated municipal sewage sludge spiked with CeO_2 of different particle sizes (4 nm ($nCeO_2$), 26 nm ($mCeO_2$) and >16 μm ($bCeO_2$)) in a pilot fluidized bed reactor (FBR) to investigate the transformation of CeO_2 under conditions mimicking full-scale incineration facilities. We used Ce K- and L_3-edge X-ray absorption spectroscopy (XAS) complemented by analytical electron microscopy to assess the speciation of Ce and to characterize the morphology of the Ce-(nano)particles. After one week of incubation in digested sludge under anaerobic condition, $nCeO_2$ already showed partial reduction to a Ce(III)-phase (~40%). Cerium dioxide of larger particle size (m- and $bCeO_2$) was less reactive and did not show any detectable reduction within the same reaction time. In general, incineration of the sludge lead to a further reduction of Ce, which was not observed in previous studies, where pure $nCeO_2$ was incinerated in a muffle oven. Reduction during incineration was nearly quantitative for $nCeO_2$ and much less pronounced for the m-and $bCeO_2$. Ce(IV) in larger (primary-) particle sizes thus was more resistant to Ce reduction during incubation and incineration. In addition, Ce-containing particles in the $nCeO_2$ fly ash showed different morphology and size compared to the original material. We therefore propose that engineered properties of nanocrystalline CeO_2 are likely lost after incineration. Our results also highlight the importance of particle size and the surrounding matrix for the transformation of CeO_2 during sewage sludge incineration.

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Long term approaches to determine the fate of ceriumdioxid nanomaterial in soil

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The increasing use of engineered nanomaterials (NM) leads to their release into the environment. There is a special focus on ceriumdioxid nanomaterials (CeO_2 NM) because of their widespread applications (diesel fuel additive, electronic and optical devices, metallurgy, polishing agents for glass and silicon wafers, exterior paints). Atmospheric deposition and sewage sludge applications have been discussed as source for the environmental release of CeO_2 NM. Sludge amendments will result in a CeO_2 NM input into arable soils, which are supposed to be a sink for NM. Here, we summarize the results about the fate and bioavailability of CeO_2 NM from a long term outdoor lysimeter study. The outdoor lysimeter study was performed with CeO_2 NM (CeO_2 NM-212, OECD Sponsorship Programme) which was applied to the lysimeter soil (Refesol 01A, slightly loamy Cambisol, OECD standard 216/217) by sewage sludge or artificial rainwater. After the application, the upper 20 cm of the lysimeters were dug up to receive comparable conditions between the different approaches. The set concentrations of CeO_2 NM after sludge application were 0 mg kg^{-1} (control), 10 mg kg^{-1} and 50 mg kg^{-1} as well as 0 mg kg^{-1} (control) and 10 mg kg^{-1} after rainwater application. The Ce concentrations of percolating water as well as the digested plant materials and soil samples were measured by ICP-OES or ICP-MS. To retrieve information regarding the Ce speciation in the lysimeter, two soil samples were investigated with synchrotron based X-ray absorption near edge spectroscopy (XANES). For both approaches (sludge and rainwater application), no vertical Ce translocation was detected over two years. The Ce concentrations in the percolating water showed no differences in Ce release between the CeO_2 NM spiked lysimeters and the unspiked controls. However, the low Ce uptake to the plant roots increasing from canola to barely suggests that despite the large CeO_2 NM retention, the NM are somehow bioavailable in the rhizosphere. The XANES results showed that Ce^{4+} was partly reduced to Ce^{3+} during the two years of CeO_2 NM aging in the lysimeter soil (50 mg kg^{-1} approach). In general, the results imply that the tested soil is a large sink for CeO_2 NM. However, information regarding the translocation and speciation of CeO_2 NM in crops are still incomplete. Hence, further investigations under realistic exposure scenarios regarding the Ce speciation at the root soil interface are required.

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Colloidal stability and bioactivity of Fe3O4 nanoparticles functionalized with humics and silica

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Investigations of Fe_3O_4 nanoparticles (NPs) functionalized by different inorganic and organic ligands are being increasingly reported in the literature due to the importance for biomedical and ecological applications. The huge diversity of polymers allows for different types of Fe_3O_4 NPs surface functionalization, i.e., introduces charges on the system that can prevent the aggregation of the particles in liquids and improve their chemical stability through surface charge control. In addition to studying the size, structure and composition of the colloidal particles, control of their colloidal stability is very important. Controlling the stability of the magnetic fluids against aggregation is important because the formation of aggregates alters their specific surface area and dispersibility as well as their bioactivity. In the present work, the influence of humic acids (HA) and/or silanes (tetraethoxysilane, TEOS and 3-amino propyl-triethoxysilane, APTES) was studied and correlated with the colloidal stability and bioactivity. The control of the functionalization nature and density (number of layers) on the Fe_3O_4 surface was investigated, electrokinetic measurements (zeta-potential and hydrodynamic diameter), bioactivity analysis were performed. The higher plant − whitemustard *Sinapis alba* L., green algae *Scenedesmus quadricauda* (Turb.) Brev., and infusorians *P?ramecium caudatum* were used as test organisms for bioactivity evaluation. The results suggest that surface charge and bioactivity can be controlled by grafting Fe_3O_4 NPs with HA and/or silanes. The sequential layer-by-layer grafting of precursors onto the NPs surface demonstrated an logical increase in the hydrodynamic diameter (measured by dynamic light scattering) in the row: Fe_3O_4/TEOS< Fe_3O_4/TEOS/APTES< Fe_3O_4/TEOS/APTES/HA. Zeta potential for silica grafted NPs varied considerably with layering from -20 mV (Fe_3O_4/TEOS) to -0.6 mV (Fe_3O_4/TEOS/APTES), which correlates with surface charge provided by the surface amino group of APTES. For the Fe_3O_4 coated by HA zeta-potential increased from 15 mV (bare Fe_3O_4) to -40 mV (Fe_3O_4/HA). The bioactivity of bare and functionalized NPs by silica and/or HA with respect to test-organisms demonstrated higher toxicity for multilayered nanoparticles as Fe_3O_4/TEOS/APTES/HA in compare with Fe_3O_4/HA. *Acknowledgement.* This research has been financed by the Russian Foundation for Basic Research (*#18-33-01270/18)* and the Russian Science Foundation (#16-14-00167).

Advances in Soil Ecotoxicology and Risk Assessment - Impact, Ecotoxicity Tests, and Concepts for a Retrospective Environmental Risk Assessment (III)

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Potential impacts of plant protection products on earthworms, carabid beetles and vertebrates on agricultural fields
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The productivity and sustainability of agricultural fields depend on the maintenance of relevant ecosystem functions as well as on the existence of a sufficiently complex species diversity. In this context, earthworms play a prominent role for soil ecosystem by influencing organic and inorganic matter breakdown, whereas carabid beetles are one of the most important arthropod predators on soil surface. So far, the environmental risk assessment of potential impacts of plant protection products on earthworms (EFSA 2017) and ground beetles (EFSA 2015) is based on single compounds and does not take into account that organisms within and above the soil are exposed to a mixture of active ingredients from different plant protection products within one season and partly over successive seasons. In addition, the potential for secondary poisoning, as assessed in EFSA (2009), may increase if contaminated earthworms and carabid beetles are combined in the diet of a mixed-diet feeding bird or mammal. In a monitoring project, earthworms and carabid beetles were sampled at two seasons from eight fields in Croatia and analysed for up to 300 active ingredients, but no soil concentrations were determined. Based on publicly available draft assessment reports from EC and EFSA, degradation parameters (DT_{50}, DT_{90}) were used to calculate degradation curves and the current soil concentration at the date of earthworm sampling as well as the current residue concentration on soil surface at the date of carabid sampling. Subsequently, bioaccumulation factors were calculated by dividing the analysed pesticide residues in earthworms and carabid beetles by the calculated concentrations in soil and on soil, respectively. The aim of this survey was to check the applicability and reliability of this method and to examine (i) whether a potential of secondary poisoning is needed to be addressed when a bird or mammal feeds both on contaminated earthworms and carabid beetles, (ii) whether the re-calculated soil concentrations of individual active ingredients and mixtures of active ingredients with the same mode of action do pose a potential risk to earthworms and carabid beetles.

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Improving risk assessment of contaminants in material spread to land
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Recovery of organic materials to land is an important source of nutrients and organic matter and also a means of minimising waste in a circular economy. However, the presence of chemicals in the recycled materials including pharmaceuticals, veterinary medicines, personal care products and industrial chemicals are becoming an increasing concern in terms of the risks to soil health, wildlife and consumers. Previous assessments of land-spreading have generally had significant deficiencies as they have had to rely on the use of exposures concentrations from the literature, often from different geographical locations, that may have little relevance to the area for which an assessment is taking place. Furthermore, the data may not relate to the particular form of material in which it is applied to land. It is therefore important to obtain data on contaminant levels in the relevant materials in order to perform accurate exposure assessment. Sound risk assessment for environmental and human health is also reliant upon robust evidence on the adverse effects of contaminants. This is often an area of weakness for terrestrial risk assessment as there is an absence of reliable (eco)toxicity data for many of the substances. A study of landspreading was undertaken on behalf of the Scottish Environment Agency (SEPA) and the Environment Agency that involved the sampling and analysis of Scottish biosolids, compost and various animal manures from England and Scotland, all in the specific form in which they are applied to land. Most contaminants were found to occur at lower concentrations than previously reported in the literature, although the synthetic musk HHCB (galaxolide) was measured at elevated concentrations in biosolids; the assessment for long term application to land indicates a potential risk to soil organisms from this substance with RCRs >1. The medicine, ciprofloxacin was identified as posing a potential risk to soil from biosolids application and RCRs>1 were calculated for contaminants in other organic materials. However, some of these conclusions need to be treated with caution as there is considerable uncertainty with many PNECs. Overall, this project indicated a limited potential for risks from organic contaminants in organic materials applied to agricultural soil but it is recommended that there needs to be a broad initiative to compile or generate the necessary data to derive robust terrestrial PNECs that are applicable across Europe and further afield.

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Scenario Development for Off-field Soil Exposure and Risk Assessment
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In its Scientific Opinion on risk assessment for in-soil organisms EFSA proposes to introduce an off-field risk assessment as soil organisms are potentially exposed to plant protection products outside the treated fields. EFSA proposes a preliminary approach for off-field soil exposure by adding up entries from the different major exposure routes (i.e. runoff, erosion, and drift depositions). EFSA indicates the worst-case character of its scenario "*In the absence of appropriate off-field exposure scenarios…*", and hence, emphasises the necessity for model and scenario development. The present work undertakes first steps (i) to develop a model approach for off-field soil exposure due to runoff, erosion, and drift, (ii) to develop exemplary schematic and real-world scenarios, (iii) which allow to gain insights in off-field soil exposure and risk using case studies. The developments are based on the Specific Protection Goals options discussed by EFSA. Results are intended to support the design of off-field soil exposure and risk characterisation scenarios in a tiered risk assessment scheme and the development of assessment endpoints (e.g. spatiotemporal percentiles of off-field exposure and risk) relevant to address SPGs, with particular focus on the identification of mitigating options (e.g. drift mitigation, vegetated filter strips, in-crop no-spray buffer). Following the developed exposure approach the off-field risk to soil organisms distributed in space and time will be presented depending on substance properties and risk mitigation options and discussed in context of possible future risk assessment framework for soil organisms. The approach is also suitabel for directly linking spatio-temporal explicit exposure with effect modelling (toxicological, population, community) for future risk assessment approaches.

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Mind the gap - On the way to a spatial and temporal explicit TKTD model for earthworms
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Soils can be considered to be among the most essential bases of human life. It is a precious resource providing food and material, but also a diverse habitat for many organisms. In this habitat, soil organisms like earthworms may be exposed to many different stressors, such as environmental or anthropogenic stressors. Chemical stress, e.g. caused by agrochemicals, is addressed in the regulatory risk assessment which aims at preventing any adverse effects on the environment and its fauna. For soil organisms, the risk assessment is based on two key elements: the exposure and the effect assessment. Principally, the more advanced exposure models can take the spatial and temporal scale of chemicals into account. The effect assessment in the lower tier considers a limited temporal or spatial variability. However, in the example of earthworms, those scales play a major role as the species move vertically and/or horizontally through the soil and may change their burrowing behaviour in response to changing environmental factors. In this regard, modelling offers a powerful tool not only to overcome the gap between exposure and effect assessment, but also to increase the understanding of earthworm ecology. Taking into account chemical effects, e.g. caused by agrochemicals, toxicokinetic-toxicodynamic models are particularly suitable to make effect predictions for time variable exposure situations since they consider the underlying mechanisms. We will outline a modular approach and the challenges that need to be faced. Further, the results will highlight the importance of including the ecology and movement behaviour of earthworms in the risk assessment. Through linking the movement of the earthworms with the exposure scenarios already in use, a realistic exposure pattern can be identified. The TKTD approach will then complete the model by assessing survival probabilities in relation to experienced concentration levels. Our results emphasize that an increased ecological understanding is necessary to bridge the gap between exposure and effect assessment in soil. The suggested modular approach combines available methods for the exposure assessment with new methods to address the effects of plant protection products on earthworms. Integrated risk assessment by using a modular model approach linking exposure and effect models will provide sound higher tier refinement options for the risk assessment of soil organisms.

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Applicability boundaries of the Equilibrium Partitioning Method (EPM) and alternative screening tools to predict toxicity to soil organisms
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According to REACH and BPR the equilibrium partitioning method (EPM) may be applied in some cases to assess the hazard to soil and sediment organisms in the absence of laboratory or field test. EPM is used to predict toxicity of substances based on results from aquatic toxicity assessment. The objective was to identify when the screening approach is adequate, what are its limitations, and in which cases further testing is needed to assess the toxicity to soil and sediment organisms. In addition, the project aimed to address the potential of other alternative screening tools within REACH and BPR. Data for this analysis was collected from the REACH registration database, and dossiers evaluated under Biocidal Products Regulation (BPR). Overall, the EPM method is protective in most cases, however, there is a clear indication that the selection of log Koc is an important factor on the accuracy of the prediction and should therefore be selected carefully. Alternative methods are, however, in their early stages and may not be applicable to all groups of organisms nevertheless could be used in conjunction to the EPM in a weight of evidence approach to support the conclusion o the toxicity to soil of a particular compound. The conclusions of this projects propose some suggestions to revise current guidelines for REACH and Biocides and identifyies areas for further development.

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Verification of the current risk assessment scheme for earthworms by empirical evidence
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The risk prediction for soil organisms in agricultural used habitats due to the application of PPPs is based in a first step on standardized laboratory tests with earthworms in combination with predicted environmental concentrations (PECs) and an assessment factor of 5. If the Tier 1 risk assessment fails, field studies may be conducted to show whether unacceptable effects on earthworms will occur in the field under realistic conditions of use. In order to confirm the current evaluation process of PPP's and their actives in terms of applied soil depth as well as the used assessment factor in the first step of the risk assessment, all available field studies on earthworms of the database of the federal agency for environment (UBA) have been analyzed for a comparison with the outcome of the first step of the risk assessment in combination with three different soil depth: 1 cm, 2.5 cm and 5 cm. The survey identified 38 field studies fulfilling the current requirements according to [5]. To investigate the current regulatory context when evaluating submitted higher tier studies, the No Observed Effect Concentrations (NOEC) determined in the field studies were compared to the outcome of Tier 1 assessment steps. For the assessment of the protectivity of the current scheme, only field studies showing effects in terms of a Lowest Observed Effect Rate (LOER) were considered for the evaluation and compared to Tier 1 outcomes as in Table 1. In this case, 22 studies fullfilled the requirements. Independently from the sorptiveness of the active substances in soil, the choice of a soil depth of 1 cm for the calculation of the PEC in the first step of the risk assessment does not lead to the under-estimation of the 'real' risk for earthworm communities in the field based on the evaluated data. However, in approx. 25% of the analyzed cases, the choice of 5 cm soil depth for the calculation of the relevant PEC leads to an underestimation of effects in the field based on the first step of the risk assessment. As a consequence, the high protection level for soil organisms and biodiversity according to [2] might not be implemented. For the planned revision of the Guidance Document on Terrestrial Ecotoxicology, a calibration of a possible risk assessment scheme will need to take not only the comparison of field to laboratory data into account but also further source of uncertainties (e.g. [6 and 7]).

Adverse Effects of Chemicals on Host-associated and Free-living Microbiomes (II)

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A novel metaproteomics approach to better assess the dynamics and functions of host-associated microbiomes from bioindicator species used in ecotoxicology
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Host-associated microbiota influences the life history traits of host animals and plays a major role in crucial apical endpoints such as survival, growth, resistance, and reproduction. However, the significance of these interactions in an ecotoxicological context has only been considered very recently. Data in the literature emphasizes the capacity of gut microbes to metabolize environmental chemicals and modulating their toxicity for the host. Because of the great importance of the interactions between the microbial community and its host, exposure to environmental contaminants may disturb this symbiosis and provoke effects in both host physiology and bacterial communities. In this work, we propose an innovative metaproteomics-based approach for microbiome analysis. By analyzing the peptide content of a given sample through tandem mass spectrometry, our approach allows for a quick taxonomic identification of the different organisms present in the sample, as well as their functional profile. The method allows identifying bacteria, archaea, yeasts, fungi, parasites, residual food and host biomarkers in one single analysis. Preliminary experiments have been done on midgut and hepatopancreatic caecum sampled from individual *Gammarus fossarum* animals. We could detect the presence of four main bacterial phyla dominating the microbiota: *Proteobacteria* (46%), *Firmicutes* (27%), *Actinobacteria* (24%), and *Bacteroidetes* (3%). More than 30 different genera could be differentiated and quantified. In addition, a global view of the main microbial metabolic pathways indicates the molecular functions associated with key processes for the host such as nutrition, digestion, and molt. Future studies will be conducted for assessing the role of microbiome dysbiosis in the health status of *Gammarus fossarum* upon different toxic stresses.

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Associations between PAH pollution and environmental and human microbiota in kindergartens
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Today, more than half of the human population lives in urban areas that typically contain polycyclic aromatic hydrocarbons (PAHs). PAH contamination can change soil bacterial community composition. Since environmental microbiota is associated with human commensal microbiota, this contamination may in turn affect immune response and health. In addition, PAHs are known to cause direct health deficits, such as cancer. For these reasons, we investigated PAH pollution and environmental and human bacterial communities in kindergartens. We measured the concentrations of PAHs from the uppermost surface soil and air of eleven kindergartens yards in two city centres in southern Finland. Bacterial communities were analysed from soil and from children's skin, Each yard was sampled on five locations. To assess potential health hazards, we used the toxicity equivalent method i.e. converted observed soil PAH contamination to benzo(a)pyrene (BaP) total potency equivalents (tpe) We also simulated bioconcentration using SemiPermeable Membrane Device samplers to measure PAHs in the ambient air. There were three air samplers in each yard. For skin bacterial community analyzes, we collected children's swab samples from back of the hand. Bacterial communities in soil and on skin were analyzed using Illumina MiSeq 16S rRNA gene metabarcoding at phylum and OTU levels. Concentrations of fluoranthene exceeded environmental threshold value in two daycare yards. Benzo[a]pyrene concentrations were above the No Observed Effect Concentration for health in one sample. When benzo[a]pyrene total potency equivalents were used, the same sample was over two times higher than the allowed threshold for incremental lifetime cancer risk. Total PAHs were above background levels in several yards. In surface soil of daycare yards, bacterial communities were associated with PAH pollution as in our earlier studies. Interestingly, concentrations of individual PAH compounds in surface soil and in ambient air correlated with children's skin bacterial communities at phylum and OTU levels. We conclude that even subtle air pollution can result in surface soil concentrations that are higher than NOEC for health. In addition to the direct health consequences, PAHs may also change the abundance and diversity of human health-associated bacteria.

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Are we blinded by the light? Increased chlorophyll fluorescence masks adverse effects in herbicide-exposed periphytic communities
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Agricultural streams receive pesticide cocktails from arable lands during or following pesticide application. Among these pesticides, herbicides have been identified as the strongest pressure for algae and should consequently induce effects on periphytic structure and functioning. Although herbicide effects on periphyton have been frequently reported, the focus has yet been on herbicides that directly affect photosynthesis. The impact of herbicides with other modes of toxic action remains largely unknown. We therefore conducted a 21-days-lasting bioassay during which periphytic communities were exposed to the carotenoid-biosynthesis-inhibiting herbicide diflufenican for 12 days (up to 10 μg/L; n=4), followed by a 9-days-lasting recovery phase in herbicide-free medium. Every third day, periphytic pigment concentrations and algal community composition were

quantified by spectrophotometry and microscopic techniques, respectively. At the same time, periphytic functioning (photosynthetic efficiency and non-photochemical quenching [NPQ]) was assessed using pulse amplitude modulation fluorometry, which measures chlorophyll fluorescence and converts it into photosynthetic efficiency. When exposed to ≥0.2 µg diflufenican/L, periphyton showed 20-30% reduced chlorophyll a and carotenoid concentrations, likely explained by an up to ~35% reduced algal biovolume as well as diflufenican's mode of toxic action that shifted periphytic communities towards a higher heterotrophy. Simultaneously, the functioning in terms of photosynthetic efficiency surprisingly increased by up to ~15% under herbicide exposure judged on higher chlorophyll fluorescence. These unexpected findings may be explained by an up to 60% reduced NPQ as well as binding of diflufenican to the pigment-protein membrane complex of the photosystem II, two processes inducing higher chlorophyll fluorescence but not photosynthetic efficiency. Although increases in chlorophyll a concentrations and NPQ indicated some recovery potential during the second phase of the experiment, the persistence of a higher photosynthetic efficiency questions a quick recovery of periphyton from herbicide stress. While the processes underlying the observed functional shifts need further scrutiny, the potential shift towards a higher degree of heterotrophy in periphyton ultimately suggests an increasing importance of heterotrophic ecosystem functions in impacted stream sites over the long term.

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Using bacterial efflux pumps as indicator of natural and anthropogenic stressors
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The efflux pumps of the two primary RND (resistance-nodulation-cell division) families are phylogenetically close together and almost exclusively found in Gram negative bacteria. The first family (HME, for Heavy Metal Efflux) includes pumps that have been described as exclusively exporting metals, i.e. mono- and di-valent cations. The second family (HAE-1, for Hydrophobe/Amphiphile Efflux-1) includes pumps involved in the export of organic molecules toxic to bacteria, such as antibiotics, solvents, bile, detergent or aromatic molecules. Due to their very wide substrate specificity, many pumps belonging to this second RND family have been described as involved in the multi drug resistance (MDR) phenotype, but also in bacterial stress response and pathogenicity. Our study aimed to compare the distribution of RND pumps according to taxonomy, origin, pathogenicity and more generally the ecology of bacteria in various environments. First, we reconstructed pump phylogeny of these two RND families from 920 genomes representative of the known diversity of Gram negative bacteria. Then, we confirmed putative ecological roles of some RND pumps by quantifying RND genes in environmental samples (qPCR) using primers targeting HAE-1 and HME families. Interestingly, we found a higher number of genes encoding HME pumps in genomes of bacteria isolated from metal contaminated environments (2.66 genes per genome vs 1.13; $P < 10^{-6}$), indicating that the HME pump gene is a relevant indicator of metal contamination in soil. More surprisingly, a higher number of genes encoding HAE-1 pumps was found in bacteria isolated from the rhizosphere compared to bulk soil (6.47 vs 4.25; $P < 10^{-6}$), while no significant difference was observed between pathogens and no pathogens in human associated bacteria. Our result raises the question of the role of root environment in the emergence of MDR phenotypes in opportunistic human pathogens. Finally, we identified a new RND clade, probably a cryptic RND family, linked to marine environment. Overall, because these results show that RND pumps are linked to bacterial lifestyles, the quantification of their gene and probably their expression could be used as indicator of natural and anthropogenic stressors.

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Effects of glyphosate-based herbicides on non-target avian taxa: development and gut microbiome
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Glyphosate-based herbicides (GBHs) are the most commonly used herbicides globally. Glyphosate has long had a reputation of being non-toxic to animals, but this has recently been challenged. GBHs have shown to influence physiology, express genotoxic and teratogenic effects, with a study focus on mammalian laboratory vertebrates. Furthermore, due to the presence of GBH-sensitive EPSPS enzyme in microorganisms, GBHs may also fundamentally influence animal gut microbiome. Developmental exposure to GBHs is likely to be more detrimental than exposure during adulthood, but this has not been thoroughly studied. Potential sex-specific effects are also poorly understood. We studied the effects of long-term exposure to a moderate dose of oral GBHs on development and gut microbiome in Japanese quail (*Coturnix japonica*) males and females. GBH and control treatments (N = 38/group) were started at 10-day old hatchlings and growth, plumage development, reproductive maturation and gut microbiome (at the age of 12 and 28 weeks) were analyzed. GBH treatment did not influence growth in either male or female quails. GBH treatment delayed plumage development especially in females. Egg production nor male sexual maturation was affected by GBHs. However, glyphosate residues were detected in eggs, and thus may influence embryo development. We expect GHBs to decrease general gut microbial diversity, and especially *Firmicutes* abundance. These results are of important value for both poultry industry – especially as currently glyphosate concentration in animal feed is not regulated – as well as wildlife and humans, to increase our understanding the multitude of GBH-related effects on vertebrates.

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Differential evolution of archaeal compared to bacterial communities rendered new biomarkers of multipollution events in Mediterranean coastal rivers
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Microbiomes are essential for the proper functioning of aquatic environments but anthropogenic stressors can lead to spatial and temporal evolutions of microbial communities and affect ecosystems fonctions and services. Assessing the environmental impact of xenobiotics in aquatic microbiomes represents nowadays a fundamental issue. Concentrations of pollutants in watercourses depend on numerous factors such as land use and rainfall events. We have demonstrated that storm events are at the origin of multipollution episodes in Mediterranean coastal rivers, which are chronically submitted to regimes of droughts and floods in these regions. In this study, we aimed at understanding how these sudden contaminant changes during flood events impact the diversity of particle-attached microorganisms of the Têt River, a major coastal watercourse in the South-East of France. Bacterial structural changes inferred through high-throughput sequencing of the 16S rRNA gene occurred at two particular moments of a major flood event: during the flow peak and during combined sewer overflows (CSOs), at which multipollution phenomena were detected by a parallel in-depth study of contaminants presence. The archaeal microbiome evolved in four distinct community groupes, which did not coincide with the four phases of the flood as for the bacterial microbiome. Nevetheless, changes ocurred from the first peak of multipollution too. Constrained multivariate analyses confirmed significant differences between communities for both microbial compartments and changes coincided with significant pollutant mixtures dynamics in tested models. *Thaumarchaeota* was virtually the only *phylum* just after the flow peak, while *Euryarchaeota* dominated certain samples during CSOs and at the flow peak. Major Archaea genera are related to the nitrogen cycle and to methanogenesis. Characteristic OTUs related to cocktails of pollutants from both domains will be highligted, constituting early biosignatures of multicontamination stress.

Novel Developments in Testing Chemicals for Endocrine Disrupting Properties (II)

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An EU-NETVAL validation study on in vitro methods to inform on thyroid disruption potential
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Significant progress has been achieved recently in the EU for the regulation of endocrine disruptors (EDs). However, there are still important gaps in the available test methods to accurately assess EDs potential of chemicals. In particular, *in vitro* methods to inform on thyroid disruption potential are still lacking. To fill this gap, EURL ECVAM launched a call to EU-NETVAL members in 2017 for participation in a validation study of a set of mechanistically informative alternative methods focusing on thyroid disruption, and is now coordinating this validation study in collaboration with EU-NETVAL laboratories and the developers of the methods. A total of 17 methods have been chosen, because they are complementary and cover the main possibilities of interaction with the thyroid signalling pathway, grouped in eight blocks: (1) central regulation, (2) Thyroid Hormone (TH) synthesis (3), secretion and transport in serum of THs (4) metabolism and excretion of THs (5) local cellular concentrations, (6) cellular responses. Furthermore, the validation study also consider relevant short term *in vitro* methods (7) integrating multiple Modes of Action, and (8) integrative cellular *in vitro* methods. The prioritisation of the methods has been done according to the following criteria: 1. At least one thyroid method should cover each of the 8 blocks 2. If possible, the methods should be suitable for high-throughput approaches 3. Methods are based, whenever possible, on the use of human-derived test systems 4. The test systems (cells, cell lines, microsomes, proteins..) availability should be taken into account Fourteen EU-NETVAL facilities have been assigned to work on the selected methods. The validation study consists of two parts: part one defines the methods and assesses their transferability and reliability; part two is focused on the overall relevance of the methods. It will comply with the recently published OECD Guidance on Good

In Vitro Method Practices (GIVIMP). For each of the methods, outline protocols will be drafted including definition of the test systems used as well as the control and reference items. Those methods which perform best will be selected for further assessment in support of EU and OECD strategies to address the potential risks to human health and the environment posed by thyroid disruptors. The validation results will also be used to inform Integrated Approaches to Testing and Assessment (IATA) for the identification of thyroid disruptors.

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Effects of perchlorate on the metamorphosis of the common frog Rana temporaria

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Amphibian populations are in decline all over the world and they are the most threatened group of vertebrates. The only amphibian species used in the OECD guidelines for the test of endocrine disrupting properties are tropical, 100% aquatic species (*Xenopus sp*.) which have a very different mode of life compared to species living in temperate areas. No other standardised tests exist and this potentially results in severe shortcomings in the risk assessment of chemicals with regard to amphibians - as pointed out in a recent report from EFSA. The EFSA report shows that the risk assessment of the effect of e.g. pesticides on amphibians is very insufficient and there is a need to include endemic, temperate amphibian species into the test guidelines. Here we examine the effects of compounds with known effects on thyroid regulation in the common frog *Rana temporaria*. The effects of perchlorate will be compared to the effects in *Xenopus laevis*. Perchlorate inhibits iodine uptake in the thyroid glands by competitively inhibiting the thyroid sodium-iodide symporter (NIS). The experimental design was as close to that of OECD TG241 as possible. Clutches of fertilised eggs were collected from a pond with at least 50 breeding frogs in early April of 2018. Fifty eggs were placed in each of 24 glass aquaria with 6 L of water in a flow-through setup. Water and stock solution of perchlorate were supplied by peristaltic pumps at a water flow of 30 L per day. Temperature was16±1 °C. Four replicate aquaria were used for each exposure concentration (12, 41, 131 & 427 μg/L) - except that 8 aquaria were used in the control group. Exposure concentrations were determined by means of LC-MS-MS (Agilent 6410) and they were very stable during the experiment. At the end of the experiment June 6-8 2018, the animals were weighed and the length of the body and legs were recorded and Gossner stage determined. One half of the animals were fixated for histological analysis of the thyroid gland and the other half was frozen for hormone determination. Data from the experiment are being processed winter and spring 2018-2019 but one of the initial findings upon the histological examination is that exposure to perchlorate results in a reduced amont of liquid in the follicles of the thyroid glands. Similar effects have been observed in *X. laevis* upon exposure to perchlorate. In conclusion, it is possible to conduct experiments with endemic European frogs reflecting the conditions of OECD TG241.

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Environmental applications for in vivo thyroid active molecules identification with the XETA (Xenopus Embryonic Thyroid Assay)

D. Du Pasquier, Watchfrog S.A.

Amphibians are primary targets in the ecosystem for environmental pollutants. Surface waters can contain a diverse range of pollutants, including Plant Protection Products, pharmaceuticals and industrial compounds. Numbers of chemicals or environmental samples have been identified to possess thyroid activities. Amphibians are particularly sensitive to thyroid disruption because metamorphosis is a thyroid-dependent processes that is fundamental for amphibians. Identification of thyroid disrupters in amphibians is therefore of high concern in the context of the decline of amphibians' populations. OECD validated already two guidelines for the identification of thyroid active molecules in amphibians: The AMA and the LAGDA. These two assays are fit for purpose for the regulatory testing of chemicals at low throughput but not to screen molecules at higher throughput or to test environmental samples that couldn't be stored or sampled in large quantities. We developed the Xenopus Embryonic Thyroid Assay to offer an amphibian screen for thyroid active molecules and environmental samples. The assay is transcriptional-based, and uses a transgenic tadpole line containing the THbZIP-GFP genetic construct to detect the activity of thyroid active molecules that work through various mechanisms. The XETA is currently being validated by the OECD and it is expected that the XETA will be approved as a test guideline in April 2019. Here to illustrate the benefit of this new tool for the hazard assessment of endocrine active chemicals we describe several applications. In the SOLUTIONS project, we assessed chemicals selected from the list of substances identified as relevant European river pollutants. Among 20 tested chemicals, the XETA identified 9 thyroid active substances. The data mining exercise highlighted for almost all positive chemicals the lack of existing data for thyroid activity in Amphibians. OCDE validation focus on using the XETA to test pure chemicals but this test could be particularly useful for the hazard assessment of effluents. During the 12 past years we applied this assay to effluents including wastewater, treated wastewater, hospital effluents, water from industrial processes. Our results using the XETA showed daily variations of the thyroid effect in wastewater, linked to economic activities and rainfall and highlighted that most WWTP effluent still contains molecules presenting thyroid activity for amphibians that are released into the environment.

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Assessment of potential endocrine disrupting properties using in silico tools - first experiences under the new EU Guidance Document

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In silico procedures have gained more and more acceptance by regulatory agencies in the last few years. These methods describe computer-based calculation models based on the hypothesis that similar structural features lead to similar effects or properties. In the recently published Guidance on the identification of endocrine disruptors under Regulations (EU) No 528/2012 and (EC) No 1107/2009, *in silico* predictions are suggested to be used to generate supporting information for endocrine modalities within a weight of evidence (WoE) approach. An overview of available software tools for predicting potential endocrine activity is given in the new Guidance for the identification of endocrine disruptors (EFSA Journal 2018). It should be noted that the application of *in silico* methods for the assessment of potential endocrine-disrupting chemicals is particularly challenging due to the inherent complexity of the endocrine system itself. While the scientific understanding of the endocrine system and testing methods for the purpose of regulatory human health and environmental risk assessments have advanced in recent years, *in silico* methods for assessing the potential of endocrine activity or endocrine disrupting properties is lagging. In this respect, it is noteworthy that among the aims of the recently initiated European research project (LIFE CONCERT REACH) in which six research institutes and consultant companies are participating, the development/integration of new models for predicting potential endocrine activities, within the VEGA software platform [www.vegahub.eu] is anticipated. QSAR models are typically restricted in their applicability depending on the chemical space in which they have been developed. Indeed, the reliability of results is highly dependent on the quality of the underlying dataset and the similarity of the assessed substance to those comprising the dataset used for the model development. In this presentation, we will address the challenges encountered when using a battery of *in silico* tools predicting various pathways for endocrine disruption. Furthermore, we will summarise our first hand experiences of using *in silico* models in the context of the application of the new Guidance for the identification of endocrine disruptors and how contradicting results have been addressed.

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A nuclear and membrane oestrogen receptor assay to test the oestrogenic activities of pollutants in fish

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Oestrogenic compounds are well known endocrine disruptors. In aquatic ecosystems, these compounds may derive from natural (e.g. phytoestrogens) or anthropogenic sources (e.g. pharmaceutical products such as contraceptives or antidepressants) and a continuous exposition to these pollutants may threaten animals' health. Oestrogens are important regulators of multiple physiological processes and oestrogenic actions are mediated across vertebrates by binding to several oestrogen receptor types. Specific transcription factors, designated nuclear oestrogen receptors (Esrs), have been mainly associated with the classical activation of gene expression by oestrogens or structurally similar compounds. More recently, membrane receptors such as the G protein-coupled oestrogen receptor (Gper) have been associated with rapid, non-genomic responses to estrogens. Both signaling pathways are present in fish, which express additional gene duplicates for both receptor types, although relative oestrogenic activities to the different receptors may differ between species. Using transient transfections in the human embryonic kidney cell line HEK293 and an ERE-luciferase reporter gene assay, the activation of sea bass Esrs was studied in response to of two compounds, the phytoestrogen genistein and the antidepressant and emerging pollutant fluoxetine (Prozac®). In addition, the activation of the two recently identified sea bass Gper duplicates was evaluated using a cAMP response element-luciferase reporter gene assay. Results indicate that genistein and fluoxetine affect each nuclear receptor in a different manner: Fluoxetine rather triggers an anti-oestrogenic response, while genistein behaves as an oestrogen mimic in the transactivation of the three Esrs. Oestradiol was able to induce luciferase activity in cells transfected with both membrane receptors, confirming, for the first time, that both teleost gene duplicates are functional. Furthermore, several xenoestrogens also stimulated luciferase activity and G1 and G15 were confirmed as agonist and antagonist of sea bass Gper, respectively. This study also demonstrates the suitability of the luciferase reporter assay as a tool to assess the oestrogenic potency and mechanisms of action of a wide variety of chemicals. Potential risks of the exposure of fish to various compounds in the environment or in aquaculture can be efficiently identified.

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Use the Right Tool for the Job! Mechanistic Potency Is Essential for Hazard Identification

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Regulatory initiatives for identifying and managing chemical endocrine disruptors (EDs) are being developed and implemented in several jurisdictions in response to concerns that exogenous chemicals may interfere with the endocrine systems of humans and environmental organisms. According to the World Health Organization's International Programme on Chemical Safety, an ED must (1) alter the function of the endocrine system; and (2) as a consequence of that alteration, cause an adverse health effect in humans or wildlife. This definition requires a causal link between the endocrine mode of action (MoA) and the adverse effect. However, with the publication of the ECHA/EFSA guidance for the identification of EDs, this requirement for a causal association appears to have shifted to one that is "biologically plausible." This is a very different criterion, especially when the guiding regulatory approach is one of hazard and not risk. Pathway models are being established that provide plausible links between molecular initiating events, key events and ultimately adverse effects. However, when several potential pathways or MoA converge on the same adverse effect in an intact organism it becomes challenging to identify the biologically plausible *causal* link between the MoA and the environmental or health effect of concern. To minimize the risk of misidentification of an endocrine MoA, an objective and transparent weight of evidence (WoE) procedure based on biological plausibility, essentiality, and empirical evidence is recommended. In order to determine whether it is biologically plausible that a chemical produces an adverse effect via an endocrine MoA, i.e, that it might fit the definition of an ED, the mechanistic potency must be evaluated to measure the strength of a chemical's activity via a specific endocrine MoA. We present a case study that applies a recently published potency threshold methodology (Borgert et al. 2018) to two chemicals identified on the Danish List of Endocrine Disrupters, a cyclic siloxane and bisphenol A. Mechanistic potency was evaluated along with other relevant information in a WoE framework to determine the potential for the chemical to meet the definition of an ED.

Improving Decision Support for Sustainability Going beyond Standard LCA (II)

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Uncertainty of product systems in LCA of emerging technologies

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With the recent drive to use LCA to assess the environmental impacts of emerging technologies, new and increased sources of uncertainty have become relevant. These are mostly related to the unpredictable evolution of the manufacturing pathways that are chosen as the technology moves from lab to industrial scale. The same applies to the multiple and/or unforeseen ways in which the technology will be used, disposed and/or recycled. Several LCA studies of emerging technologies have opted to address these uncertainties by assessing different scenarios which allow practitioners to make if/then types of conclusions. However, multiple if/then conclusions can only offer limited guidance for policy decisions that must be made with the current state of knowledge. The scenario-based approach also brings a problem of a more practical nature; considering all the different possibilities, the number of scenarios can quickly become unmanageable. We present a framework in which possible future configurations of a technology's product system are assessed as a single product system with uncertain components. The uncertain components are unit processes in the manufacturing, use, and/or end-of-life stages which may or may not be used in an industrial scale implementation of the technology. For this we set up a product system that includes all alternative unit processes, and then randomly activate or deactivate them according to defined probabilities of occurrence. This method allows us to run a Monte Carlo simulation on the single product system to obtain a single probability distribution for each impact category. The resulting distribution accounts for uncertainty about the product system's final configuration, along with other sources of uncertainty e.g in the background systems or other modelling choices. Our framework provides a way to investigate whether an emerging technology is likely to outperform the incumbent technology. It distinguishes itself from scenario-based approaches, which are better suited to identify which of the possible scenarios are preferable from an environmental point of view. Our approach is more adequate for a situation where there is no actual choice, rather uncertainty about which processes will enable the technology in the long run, which is often the case. We therefore advocate for a clear distinction of the goal of the LCA in each case, and use of the appropriate framing to achieve it.

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Prospective inventory modelling of emerging chemicals: The case of photonic materials

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Prospective life cycle assessment (LCA), or ex-ante LCA, has been defined as an assessment of a product system modeled at a future time, before its commercialization. Such assessments bring the promise of altering emerging technologies in a more environmentally benefitial direction before they become difficult to change. Since the future cannot be known with certainty, prospective modeling need to rely on scenarios of various kinds. However, how to conduct such prospective scenario modeling in practice still has to be clarified. In this study, we have modeled two emerging chemicals that can be used for a technology called photon upconversion, which converts low-energy light into higher-energy light harvestable by solar photovoltaics, thereby increasing their efficiency. Two chemicals currently considered for this purpose are ruthenium bipyridine chloride (RBC) and diphenylanthracene (DPA). These novel, emerging chemicals have not been studied regarding environmental performance before and are consequently not present in any LCA databases. The aim of this study is to present a generic procedure for prospective inventory modeling of emerging chemicals and apply that to the cases of RBC and DPA by developing unit processes for these two chemicals. An industrial synthesis scenario was adopted as our main scenario, reflecting a possible future time when RBC and DPA are produced at an industrial scale. The modeling was conducted in six steps: (1) Identify likely chemical syntheses. (2) Calculate inputs stoichiometrically based on the chemical synthesis reactions. (3) Modify inputs based on available yields for reactants and solvents (e.g. obtained from patents or estimated). (4) Categorize outputs as by-products or waste depending on their likely subsequent fate. (5) Calculate process emissions. (6) Model energy flows. Unit processes for the two emerging chemicals are thusly developed. The procedure is considered particularly strong for estimating inputs and output materials related to the stoichiometric reaction, but weaker regarding the estimation of emissions and energy requrement. Further research into the modeling of energy flows for high-temperature processes is therefore recommended, as well as estimation procedures for emissions from emerging chemicals production.

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Ex-ante life cycle assessment of the cumulative energy demand of a perovskite solar cell

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The perovskite solar cell (PSC) has shown an outstanding evolution from power conversion efficiency of 3.8% in 2009 to 23.3% in 2018 [1] and is the most promising emerging photovoltaic technology (PV). However, from a life-cycle point of view, other emerging PVs with lower maximum efficiencies such as organic PV (OPV) with 12.6%, seem to be more promising. From a review of the respective publications, however, it can be shown that the OPV impacts results are drawn from ex-ante LCAs of prospective OPVs, whereas the PSC impacts results are based on existing lab-based data in ex-post LCAs. We conclude that there is a connection between higher cumulative energy demands (CED) and overall higher environmental impacts due to lab-data, whereas CED of commercial production can be expected to be considerably lower. To verify this assumption, we developed a novel approach to scale up the CED of PSC manufacturing processes. In the following, we present our approach and compare our CED results to recent LCA studies on PSC and OPV. We differentiated three upscaling scenarios A), B), and C) whereas the CED of A) is reduced by 50% to B) and by 80 % from B) to C). The CED of A) is comparable with the PSC literature estimates since we only applied linear upscaling without scale effects. Our calculated CED is lower than the median of the PSC literature what might result partly from the unknown processes but also from differences in the considered embedded materials and manufacturing processes of each reviewed LCA study. Scenario B) and C) are comparable with the OPV literature since both scenarios integrates scale effects considering that manufacturing processes of industrial PSCs run more efficiently than of lab-scaled. In scenario B), this efficiency is displayed by the integration of process-specific scaling factors. Scenario C) is independent from the lab-data and has the lowest CED impacts. That is not the case in general but results from inefficient lab devices which affect scenario B) but has no impacts on C). In case of devices with efficiencies above the average, scenario B) would lead to higher scale effect and result in lower CED than scenario C). The presented approach can be applied to get more realistic results of ex-ante LCA for commercial production of prospective PSC. Furthermore, the approach can be easily transferred to other emerging technologies and support the performance of ex-ante LCA at a very early stage of technology development.

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Integrated ex-ante environmental and techno-economic assessment of plasma gasification for enhanced landfill mining

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When addressing new waste management systems, an in depth analysis of the possible system design alternatives and their environmental and techno-economic performances is required to support decision making. New approaches are needed for early stage assessments of emerging waste management technologies to address the uncertainties associated to alternative system design concepts, upscaling and lack of data. In particular, it needs to account variability of site-specific conditions such as different waste feedstock, uniformity of scale among (sub)processes, and limited primary data due to lack of commercial implementation. Results from environmental and economic assessments could then be more reliable for supporting decision making. This study addresses the potential of plasma gasification as combined material and energy valorisation technology for exacavated landfill waste in the context of enhanced landfill mining (ELFM). Main products of plasma gasification are a high quality syngas, metals and a vitrified slag which can be used for the production of new construction materials. However, plasma gasification is still an emerging technology which has mainly been studied at pilot scale. Different design alternatives should be considered when assessing its feasibility at commercial scale, including feedstock composition, gasifying agent, etc. The goal of the study is to understand which critical factors mostly influence the techno-economic and environmental performance of the process and how their variation can affect the outcome, and under which conditions the technology would represent a viable solution for combined energy and materialrecovery. The ex-ante integrated economic and environmental assessment is conducted through a factor based-approach and multiple-scenario analysis. 8 critical performance factors are identified for plasma gasification at a system, project and site-level. For each factor, 3 alternative datasets are considered. A factorial number of scenarios (3^8=6561) is built as unique combination of the factors and their datasets The scenarios simulate alternative technology designs and ranges of performances. Equilibrium models are then applied to calculate mass and energy balances for the scenarios and address the upscaling issues related to the assessment at industrial scale. A variance based sensitivity analysis is eventually conducted to identify the most relevant factors in the techno-economic and environmental performance.

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Challenges of prospective life cycle assessment of emerging cell-culture based technologies for food production

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Due to the environmental challenges related to agriculture and livestock production, researchers have started to develop cell-culturing based technologies for producing food and materials (i.e. cellular agriculture). The products of cellular agriculture are divided in two groups: cellular products and acellular products. Cellular products consist of the actual cells that have been cultured, whereas acellular products are proteins or other substances that are synthesized by the cultured cells - generally microbial cells. Cultured meat (i.e. in vitro meat, clean meat or cell-based meat) is an example of a cellular product, and it consists of livestock muscle cells that are cultured in a nutrition medium in a bioreactor. The range of acellular products is wide, including proteins, fatty acids and many other substances. A few prospective life cycle assessment (LCA) studies have estimated the environmental impacts cultured meat, but peer-reviewed LCA studies of the acellular products used for food are currently missing. This paper discusses the challenges of carrying out prospective LCAs for applications of cellular agriculture. Three products of cellular agriculture are used as case studies: i) cultured meat, ii) egg albumin synthesized by a fungus and iii) microbial single-cell protein. The level of challenges to perform an LCA vary depending on the product. Cultured meat had the highest uncertainties due to scarcity of knowledge of the design of large-scale production systems. The results of the previously published prospective LCA studies of cultured meat differ substantially depending on the whether they are based on currently available technologies or assume the use of possible future technologies. The second technology, egg albumin synthesized by a fungus, was less challenging due to existence of similar technologies that currently operate in large-scale production. (i.e. production of enzymes). Therefore, the main challenges were related to the assumptions of the similarity of processes (e.g. protein yields and composition of nutrition medium) access to data due to confidentiality reasons and availability of LCA data for the inputs. Regarding the microbial single-cell protein, the main challenges were related to the low technology readiness level, and therefore, difficulty to estimate the level of technological development related to the process itself and supporting processes (e.g. energy generation by solar panels and hydrolyses process).

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Poster spotlight

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Assessment of Social Impacts for Decision-Making Processes and Communication

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A critical review of impact assessment in socio-economic analysis of REACH restriction dossiers

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Under the European chemicals legislation REACH, regulatory authorities of EU member states or the European Chemicals Agency (ECHA) can submit proposals for adopting measures that restrict the use of substances posing an unacceptably high risk to society. Such restriction dossiers usually include a socio-economic analysis (SEA), providing detailed information about the expected positive or negative impacts of one or more risk management options (RMOs). REACH guidance documents suggest to assess different types of impacts, i.e. economic, environmental, human health, wider economic, social, and distributional impacts. Comparing the impacts of one or more RMO(s) against impacts of a business-as-usual scenario (i.e. impacts without a restriction) supports decision-making on the selection of the preferred RMO. Conducting an impact assessment in a REACH restriction proposal is time- and resource consuming. Obvious questions are, therefore, how impact assessments in REACH restriction dossiers were conducted, what results were obtained, and how these results contributed to decision-making. This study offers a systematic and critical evaluation of the impact assessment in REACH restriction dossiers. First, we identify the types of impacts assessed. Second, we evaluate the methodological approaches used for assessing impacts. Third, we compare the outcomes of impact assessment across dossiers. Particular attention is given to the expected risk reduction capacity of the suggested RMOs, their expected costs, and their expected (quantifiable) benefits. Finally, we analyse whether the outcomes of impact assessment systematically differ between chemicals belonging to Substances of Very High Concern (SVHC) and those which are non-SVHC. Our findings underline that impact assessment in SEA in REACH restriction dossiers is not based on a holistic approach. Furthermore, assessments have focused on selected impacts only, particularly on economic and health impacts. The assessment of economic impacts has been focusing on direct costs (compliance and substitution costs) only, but has largely ignored external costs. Hence, results from impact assessment do not warrant conclusions about the social impacts resulting from a proposed restriction. While this can sometimes be explained by the very specific scope of a restriction dossier, we also observe a lack of approaches for assessing environmental, health, social, and distributional impacts from chemicals use.

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Communicating carcinogenicity findings for layman: How much safer will painting be after TiO2 carcinogenicity classification?

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The decision-making processes related to the implementation and application of REACH and CLP-regulations aim at *ensuring a high level of protection of human health and the environment*. Tools of these regulations range from bans on substance use to warnings on substances to users. Evidently, bans define new rules for a business around a banned substance and, hence, cause social impact. However, even less severe changes in the regulatory status of a substance will have impacts on the substance use, business, and society. Current process on the classification of titanium dioxide (TiO_2) as a carcinogenic substance provides a topical example of a regulatory process. The proposed classification does not ban the use of TiO_2, but sets new requirements on hazard communication regarding the substance. Our aim was to assess impacts of the new classification on the whole product life cycle from raw materials to products and finally to the end of life stage. We used a stepwise approach in which we studied the process from the hazard assessment results to the expected goal of improved chemical safety in five steps. First, we summarized the findings on current TiO_2 exposure. Second, we compared theTiO_2 hazard assessments outcomes. Third, we studied ECHA's hazard classification process on TiO_2. Four, we searched relevant (for TiO_2) guidance tools, which refer to the hazard classification, such as eco-labels, product regulations, recommendations for purchasing, and company policies. Finally, we compared exposure to TiO_2 with carcinogenicity classification and the original exposure throughout the product life cycle. Our conclusion was that the classification of TiO_2 as a Carc Cat 2 substance would not have an impact in exposure and related chemical risks. From communication point of view, we saw a challenge in attaching standard health hazard warning in liquid products, such as paint, when the potential hazard is associated with dry TiO2 particles. On the other hand, we question the message of warning consumers on carcinogenicity in cases in which authorities have challenges in the interpretation of the toxicity assessment results. Is the intended communication with the warning: "We don't know for sure - Use at your own responsibility"? We underline that there is a risk for inflation of hazard communication along with the increasing prevalence of warning labels.

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Revision of the Guidelines for Social Life Cycle Assessment of products - status and progress
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Lots of efforts have been undertaken in the last ten years to further development the Social Life Cycle Assessment (S-LCA) methodology. It is defined as the assessment of positive and negative social impacts of a product along its life cycle. The economic crisis and the globalized market led to the necessity to more transparent supply chains and to measure the impact generated by a product/ company on the different stakeholders involved in the supply chain. The first S-LCA Guidelines were published in 2009 by the UNEP/SETAC Life Cycle Initiative. Those were complemented by the related Methodological Sheets in 2013 (UNEP, 2013). Since the launch of the 2009 and 2013 publications, many S-LCA case studies have been conducted, and methodological improvements, i.e. the special issue on Social life cycle assessment, (Macombe et al 2018) have taken place. At the same time, similar initiatives from the business world were developed: e.g. development of the Handbook of Product Social Impact Assessment (2018) released by the Roundtable of Product Social metrics in different versions (2016, 2018). Building on the existing literature, research activities and initiatives, experts in the field joined the "Social LC Alliance" effort to review the S-LCA Guidelines 10 years after its original publication by bringing a critical update. This Social LC Alliance project started in September 2017 as a flagship activity of the 5th phase of the UN environment Life Cycle Initiative. The project consists of two phases: 1) revision of the Guidelines and 2) Implementations of the new version of the Guidelines throughout new case studies. The process takes place also in cooperation with the 10YFP Consumer Information Program. The review process undertaking includes experts from all sectors (academia, business, government, NGOs,) working in small groups by topic. A comprehensive peer review process will be launched in 2019. The new capability-development format will support broadening the practice by making the Guidelines a cornerstone reference for anyone wanting to conduct a S- LCA, Social Organizational Life Cycle Assessment, Social Hotspots Assessment or a Human Rights Due Diligence Assessment. An overview of the new S-LCA Guidelines draft will be given with the description of the new aspects included as well as a description of the consultation process and inputs received to finalize the Guidelines.

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Stakeholders Categories for S-LCA application in social business: People T&C case study
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The interconnection among the three sustainability pillars (people, planet and profit) has been underlined over the last time. Several studies and research refer the sustainability to the political and cultural dimension of societies. In the S-LCA context, it is possible to say that, a society is developing socially and economically within an environmentally conditioned context and this development is directed dependent by the strategical and political organization (Mazijn, 1994). Among the activities of the joint research project between the Department of Business Studies and People Srl. with the aim of developing an innovative model to stakeholder's management based on participatory approach to improve and develop territorial networks, after a preliminary internal survey, the stakeholder categories and sub categories was defined in order to implement Social Life Cycle Assessment framework. Social Life Cycle Assessment is a technique available to account for stories and inform systematically on impacts. May it help stakeholders to effectively and efficiently engage to improve social and socio-economic conditions of production and consumption (Unep, 2009). People T&C is a consulting and training company based the core business in the values of social enterprise. The direct customers are all those who are interested in promoting organizational models based on cooperative culture and social responsibility. This work aims to define the Stakeholder Categories and Sub-categories for the social business through a case study application in People T&C. The results will be useful for the preparation of an effective business model and strategic business plan aimed at developing the company's core activities based on training and consulting services. After a literature review, a preliminary survey based of questionnaire to the internal stakeholders was constructed and conducted. The analysis was repeated for all stakeholders' categories, defined for each of the impact categories and, through network analysis support, we obtained the stakeholder map and their relationships. This stakeholder categories characterization helps to conduct the S-LCA analysis and contribute for a generalized model for similar companies.

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TBD

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Resilience, Society, and Sustainability through Equity
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From physics we know that achieving absolute sustainability for any society on Earth is not possible; there will always be material losses (waste) and energy transformations (increased entropy, degraded exergy), so by definition fossil fuel-based societies are not sustainable. But, we can always consider sustainability as a goal, however thermodynamically unreachable. Thus, any society can have the objective of achieving a level of resilience such that the planet doesn't mind their use of its ecological goods and services: a state of nominal if not absolute sustainability, and close enough to the goal. So the objective, the focus, of human societies is resilience. Not engineering resilience – the type of 'we will rebuild' resilience that increases GDP but does not solve the underlying reason for having to rebuild once, twice, thrice, perpetually. No, the type of resilience a society should strive for is ecological – that is, unremarkable, responsive, flexible, adaptive. Unremarkable in that it just happens, there is no call for meetings, no need for banner headlines. Responsive in that as occurs a change in the rate of ecological goods and services entering a social system from its supporting landscape, there occurs a timely and equivalent change in the rate of demand and use of those goods and services by society. Flexible in that as ecosystem changes occur there are no societal losers nor societal winners as a result – social systems provide for individuals and groups and industries and cities when the provision of ecological goods and services is altered. And more particularly, adaptive in that as humans occupying a landscape are presented with novel rates and volumes of provisions from a once-stable ecosystem, including the total loss of one or many goods or services, social changes and economic reorganizations occur with minimal turmoil and a new social-ecological system emerges. We will have focus on responsive, flexible and adaptive societies, communities, and tribes, with a focus on their sustainability narrative (from the four questions: what; for whom; how long; at what cost) and their perspectives on the use of science in social – ecological systems.

Organic Micropollutants in Urban Waters (II)

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Enhancing the removal of emerging micropollutants through a biological wastewater treatment at low temperatures
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Growing concentrations of emerging micropollutants (EMs) such as over-the-counter drugs, hormones and antibiotics in the environment has recently bacome a global public health issue. Many of these substances are able to pass through all the treatment steps of common municipal wastewater treatment plants (WWTPs). Consequently, these pollutants have been detected in micro-concentrations in the environmental waters all over the world. The toxic effects on the aquatic organisms, spreading antibiotic resistance and accumulation due to constant consumption of these substances are calling for the new treatment solutions. In this study, the removal rates of widely-used EMs ibuprofen, diclofenac, carbamazepine, estrone, 17β-estradiol, 17α-ethynylestradiol were studied at common process temperatures for Nordic countries (8-12°C). Data obtained from full-scale processes were compared with the results from pilot Sequencing batch reactors and Membrane Bioreactors. Different operational conditions and dynamics of microbial communities of activated sludge were studied in order to find the preferable strategy for EMs removal in cold regions. In addition, the effect of environmental concentration of antibiotics sulfadiazine and trimethoprim on activated sludge performance was studied. The results showed surprisingly lower removal rates compared to the most of the data from other countries. The main suggested reason is the strong limiting effect of low temperatures on bacteria and other microorganisms, which are commonly used in treating municipal wastewaters. Additionally, studies of activated sludge solid phase demonstrated that EMs might accumulate in bacterial cells to a large extent. Furthermore, activated sludge microbial communities were unexpectedly different from common activated sludge communities reported which affected vulnerability of microorganisms to environmental concentrations of EMs especially, antibiotics. At the same time, the research showed, that optimization of the existing treatment technologies can significantly improve the removal of several studied substances and reduce negative effects of EMs on activated sludge key microorganisms. Additionally, process configurations may affect the growth of low-temperature adapted microorganisms. Overall, the study proves that low-temperature conditions should be considered as an important parameter in modern wastewater treatment plant design.

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Biodegradation of pharmaceuticals in post-treatment moving bed biofilm reactor (MBBR): enantioselectivity and controlled co-degradation

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Moving bed biofilm reactors (MBBR) have been suggested to remove pharmaceuticals from wastewater, as a post-treatment. To overcome the scarcity of biodegradable carbon in wastewater effluents (post-treatment), a range of acetate (0-320 mg C/L) was dosed into MBBR to study the effects of acetate on biodegradation of 26 pharmaceuticals (β-blockers, analgesics, antidepressants, antibiotics and contrast media) at wastewater relevant concentrations. Reaction kinetics and enantiomeric fractions were analyzed. The results showed that all tested β-blockers and one metabolite (metoprolol acid) were degraded enantioselectively, and the enrichment of *S*- or first eluted enantiomer was observed. However, non-enantioselective degradation dominated until the majority of β-blockers was degraded, and enantiomer fractions started to change successively. This suggested the biodegradation of β-blockers was performed by both enantioselective and non-enantioselective enzymes. In the end of reaction, high doses (90-320 mg C/L) of acetate lowered the enantiomeric enrichment of atenolol, metoprolol, propranolol and metoprolol acid compared with the one with low doses of acetate, which indicated that the additional substrates like acetate could make a difference on the enantioselective biodegradation. On the other hand, the impacts of acetate on the degradation kinetics of pharmaceuticals were dose- and compound-dependent. While the removal of venlafaxine, tramadol and ciprofloxacin were promoted by dosed acetate, dosing acetate (especially with high dosage) inhibited the degradation of acetyl-sulfadiazine, ibuprofen, citalopram and diclofenac probably due to different mechanisms. Degradation of seventeen out of twenty-five tested compounds were found to be uninfluenced by the acetate dosage. These findings do not only provide evidence on dosing additional acetate cannot up-regulate removal of pharmaceuticals in post-treatment MBBR, but also help to understand relationships between easily degradable carbon source and the key biodegradation processes of pharmaceuticals.

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Biological transformation of fexofenadine and sitagliptin by carrier attached biomass and suspended sludge

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Biofilm reactors are used since the early 2000s to improve nutrient management, e.g. nitrification and denitrification, at full-scale wastewater treatment plants (WWTPs). It is assumed that the carrier attached biomass in moving bed biofilm reactors (MBBR) is much more effective in removing organic micropollutants from wastewater compared to activated sludge treatment. However, the formation of transformation products (TPs) in MBBRs, which are likely to occur, is not yet well reported. In this study the antidiabetic agent sitagliptin (STG) and the antihistaminic drug fexofenadine (FXF) were investigated concerning their behavior either in contact with suspended sludge or carrier attached biomass. Therefore, aerated batch experiments containing suspended sludge or carriers (both diluted with WWTP effluent) from a bench-scale MBBR were conducted. STG is almost completely degraded in both systems within 3 days (d). In contrast, FXF is removed much faster in contact with carriers (96% removal after 3 d) compared to suspended sludge (80% removal within 7 d). However, it can be assumed that the degradation of FXF and STG is biologically induced, as the concentration of both compounds remains stable in autoclaved carrier systems. In total, 11 TPs (7 TPs for STG and 4 TPs for FXF) were observed and chemical structures were proposed via high-resolution mass spectrometry (LC-QToF-MS). The structures of 3 TPs of STG and 2 TPs of FXF were unambiguously confirmed with available reference standards. Major transformation reactions consisted of dehydrogenation, amide cleavage or N-acylation. The formation of one major TP of STG was slightly higher in systems containing suspended sludge (reaching 55% (carrier) and 73% (sludge) of the initial STG concentration within 3 d). In contrast, the increase of one major TP of FXF was much faster in contact with carriers (57% (carriers) after 1 d compared to 58% (sludge) of initial FXF concentration after 7 d). Additionally, the TP is further degraded during the following incubation, which cannot be observed in systems with suspended sludge. This supports that carrier attached biofilms of MBBRs can improve the transformation of certain micropollutants as well as their primary TPs. Current investigations focus on the quantification of the identified TPs, in order to investigate i) their fate in advanced wastewater treatment technologies (e.g. ozonation) and ii) their relevance for the aquatic environment.

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Removal of organic micropollutants and nanoparticles in pilot scale ultrafiltration and GAC-filtration

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Municipal wastewater treatment plants, WWTPs, realeases organic micropollutants and nanoparticles to the environment. Treatment plants today can seldom control the emissions of these compounds. Upgrading WWTPs with advanced treatment methods reduces emissions and increases the possibilities to reuse water. This study aims at investigating the potential of ultrafiltration (UF) and granular activeted carbon (GAC) filtration in long-term (one year) pilot experiments treating effluent wastewater, as well as tracking organic micropollutants through a conventional WWTP. Organic micropollutants were measured with UHPLC-ESI-MS/MS. Nanoparticles were analysed before and after the pilot using Nanoparticle Tracking Analysis (NTA). The emissions of organic micropollutants and nanoparticles from the WWTP were substantially reduced with UF and GAC for the duration of the study. The ultrafiltration showed a minimum of 2.5 log reduction of nanoparticles, the effluent concentration was below the quantification limit. Organic micropollutants was predominantly removed through adsorption to the activated carbon. Eighteen of the 23 analysed compounds showed a removal efficiency higher than 95% during the first 10 000 BV. Further through the study, a majority of the compounds were removed to more than 80% throughout the experimental period, 18 000 BV, e.g. carbamazepine, tramadol, the hormone estrone and the antibiotics clarithromycin, erythromycin and trimethoprim. The antianxiety drug oxazepam, the antidepressant drug venlafaxine and the painkillers diclofenac, ibuprofen and naproxen showed a slightly lower removal (75%) be the end of the study. While most of the micropollutants were removed to a high degree throughout the study, the removal of a few compounds such as sulfamethoxazole, PFOS and flukonazol decreased substantially over time (5-56%). The study was also tracking the organic micropollutants through the WWTP with biological and chemical treatment. A few compounds were removed to more than 80% in the conventional treatment; ibuprofen, naproxen, estrone and sulfamethoxazole. All other measured compounds showed a removal of less than 25%. The emissions of organic micropollutants via municipal wastewater can be efficiently reduced through GAC-filtration. In addition, microorganisms and nanoparticles can be removed with UF. Both techniques are viable alternatives for full-scale implementations.

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Effect of ozone dose on the formation and removal of transformation products of pharmaceuticals during ozonation of secondary effluent

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Removal of organic micropollutants (e.g. pharmaceuticals) in wastewater treatment plant requires advanced treatment technologies like ozonation, activated carbon, membrane bioreactor, etc. Among several techniques, ozonation has gained popularity due to its competence to remove wide range of micropollutants also in relatively short time. However, the removal process does not simply mineralize the micropollutants rather it transforms them to other compounds. These transformation products might have adverse effects on the aquatic ecosystem and necessitate further research. Consequently, the formation of the transformation products (TPs) were quantified in dependence of the specific ozone dose in a pilot plant which was operated poststream of an activated sludge wastewater treatment plant. Samples were analyzed to quantify 17 different TPs along with various water quality parameters. Those TPs that are formed by human metabolism were removed in dependence with the specific ozone dose (mg O_3 / mg DOC). However, the typical ozonation products i.e. Venlafaxine N-oxide and Tramadol N-oxide are formed during ozonation with increasing ozone dose. The formation occurs at relative low specific ozone dose (typically around 0.5 mg O_3 / mg DOC). However, most of these ozonation products are removed again at higher specific ozone dose (typically > 0.8 mg O_3 / mg DOC).

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Removal of organic micropollutants by activated carbon - gaining mechanistic insights by thermogravimetric analysis

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Advanced wastewater treatment by the application of activated carbon is used to eliminate organic micropollutants in large scale wastewater treatment plants. However, underlying adsorption processes are unclear. On the one hand, compositions of wastewater differ and are very complex. On the other hand, activated carbons have physicochemical properties which impair investigations on adsorption mechanisms, since they are non-graphitic, non-graphitizable and have a highly disordered microstructure. This leads to their large surface areas and internal pore structure but also implies heteroatoms and functional groups. These are not easily accessible for research but affect the adsorption of organic micropollutants. Thermogravimetric analysis and evolved gas analysis can provide valuable insights into these kinds of systems. First, pyrolysis makes functional surface groups accessible, e.g. through decarboxylation, which facilitates characterisation of activated carbons. Second, adsorbates can be investigated by thermal desorption or decomposition, depending on the sorption conditions of the organic micropollutants. Single component systems as well as multi component systems with competing organic background matter are compared for various activated carbons. Surprising distinctions in the adsorption mechanisms will be presented.

The Fate, Effects, and Mitigation of Oil Spills on Aquatic and Marine Environments

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Responses of freshwater vertebrates and their food web to experimental spills of diluted bitumen
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Oil spills in inland waters may be infrequent, but their consequences on ecologically sensitive ecosystems could be profound and require further study. As part of the BOREAL (Boreal lake Oil Release Experiment by Additions to Limnocorrals) study, we conducted a series of oil spill simulation experiments at the IISD-Experimental Lakes Area, a unique freshwater research facility situated in Ontario, Canada. A pilot study was performed in 2017 over 12 days using three microcosms (2-m diameter) floated in land-based tanks, and an in-lake study was performed in 2018 over 100 days using nine limnocorrals installed in the littoral zone of an oligotrophic boreal lake. Diluted bitumen, a viscous unconventional oil product commonly transported in Canada, was used for both experiments. The specific objectives of this paper were (i) to assess the responses of different levels of the lower food web, including bacteria, phytoplankton, and zooplankton, to experimental dilbit spills; and (ii) to examine the toxicity of lake water contaminated by experimental dilbit spills to three freshwater vertebrates. We used a multifaceted approach with numerous traditional and advanced techniques to examine changes in the biomass, species composition, and diversity of prokaryotes and eukaryotes in response to a comprehensive range of dilbit spill scenarios. To evaluate toxicity of naturally weathered dilbit, we exposed fathead minnows, finescale dace, and wood frogs to lakewater contaminated by dilbit spills collected from the limnocorrals and assessed survival and a suite of sublethal endpoints including growth, development, and behaviour. Based on a pilot study performed in aquatic microcosms containing natural lake water and sediment, we conclude that, at least in the short-term, dilbit spills appear to alter the abundance, diversity, and composition of planktonic communities. Data from our in-lake study will provide important insights regarding the chronic effects of dilbit on littoral communities of boreal lakes, as well as the toxicity of naturally weathered dilbit on fish and amphibians. Preliminary data suggest a concentration-response increase in the rate of malformations for fathead minnow larvae with dilbit weathering. We anticipate this research will improve the design of sampling programs to monitor impacts and recovery of dilbit spills into inland waters from pipeline ruptures and train accidents.

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Effects of the water accommodated fraction of North Sea crude oil on Calanus finmarchicus
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Calanus finmarchicus is an ecologically important species in the North Atlantic. Due to continued development of areas for oil and gas production, as well as increase shipping, there are both continuous and accidental environmental discharges of petroleum oil in this region. The present study aimed at investigating oxidative stress, lipid peroxidation and protein damage biomarkers and changes in metabolome profile in *C. finmarchicus* exposed to a water accommodated fraction (WAF) of a naphthenic crude oil from the North Sea (NS). Non-ovulating adult females (n=5580) of *C. finmarchicus* from a continuous lab were used in exposure studies with WAF of a naphthenic NS crude oil. Individuals were exposed to a sub-lethal concentration of WAF corresponding to the 50% of the 96h LC_{50} and were sampled at 0, 24, 48, 72 and 96 hours. Standard analyses for semi-volatile (SVOC) and volatile organic compounds (VOC) in crude oil and WAF were performed with GC-MS and purge and trap GC-MS, respectively. Biomarkers of oxidative stress, lipid peroxidation and protein damage were determined using qPCR, enzymatic activities analysis and oxidative stress-related molecules malondialdehyde (MDA) and glutathione were analysed by colorimetry and UV-VIS spectrophotometry. Untargeted metabolomics using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was applied for studing effects of exposure on metabolome profile. Significant induction of glutathione S-transferases and increase of MDA concentrations in the exposed group indicates that both biomarkers are usefull indicators of oxidative stress in copepods exposed to NS crude oil WAF. In addition, the results suggest that lipid peroxidation is a main toxic endpoint in the exposed animals. In contrast, gene expression results showed inconsistent responses with both up- and downregulation. The concentrations of many metabolites (mostly amino acids), were significantly lower in the WAF exposed animals than in the control animals. Similary, the organic acid malonate concentration decreased during the experiment in control animals, but increased in the WAF exposed animals. These changes could be explained by the combined effects of oil and starvation. Metabolome changes suggest that exposure to WAF might impair energy balance in *C. finmarchicus* and malonate seems to be a promising biomarker of oil exposure. Field studies are needed to confirm and test the proposed biomarkes and metabolome changes in real scenerio of oil spil.

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Using transcriptional responses in zebrafish embryos to characterize biomarkers for crude and dispersed oil toxicity
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In this study zebrafish embryos were exposed to a naphthenic North Sea crude oil characterized by a high proportion of low molecular weight saturates and aromatics. A third generation dispersant that can be used as a chemical response action to combat oil spills was integrated. Especially during the embryonic development aqueous compounds of crude oils lead to adverse effects at low concentrations. Of major interest is the underlying molecular pathway for the affected biological functions. The results from whole transcriptome analysis provide an initial understanding of the underlying processes leading to the disruption of biological functions and can be used to focus subsequent studies using mechanism-specific bioassays. Zebrafish eggs were exposed shortly after fertilization to selected concentrations (EC_{10}) of low energy water-accommodated fractions (LEWAF) and chemically enhanced water-accommodated fractions (CEWAF). Embryos were incubated at 26°C with daily medium exchange. At 119 hours post fertilization the exposure was terminated. To investigate larval swimming behaviour a light/dark transition test was conducted using a DanioVision observation chamber and EthoVision tracking software (Noldus, Netherlands). For the RNA sequencing the Illumina NextSeq 500 was used. The cut-off value for regulation of genes used for functional annotation analysis was set to 1.5 fold greater or lesser compared to the control. The statistical analysis of expression in mRNA levels between control and treatment groups found significantly different (p value = 0.01) expression of 2223 genes for LEWAF and 4048 genes for CEWAF exposure, respectively. Among the most regulated genes in both treatment groups were genes that belong to the phase 1 metabolism. Furthermore, the analysis showed that multiple genes associated with the development of the eye and genes associated with phototransduction were regulated. Interestingly, exposed embryos showed significantly reduced swimming activity compared to control fish in dark stimulus conditions of the behavior assay. The exposure to LEWAF and CEWAF led to significant changes in expression of genes that are associated with the regulation of the circadian clock in the embryos. Hence, the present study allows the confirmation of known affected pathways that had been described for other species after exposure to crude oils but also novel pathways for crude oil mediated toxicity like the alteration of the circadian rhythm might be derived

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EFFECT OF SEASON-DEPENDING ECOLOGICAL VARIABLES ON THE RESPONSIVENESS OF A BATTERY OF BIOMARKERS IN MUSSELS (Mytilus trossulus) FROM TWO LOCALITIES IN THE BALTIC SEA
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For reliable mussel monitoring programs based on biomarkers, relevant baseline values and their natural variability need to be known. The Baltic Sea exhibits high inter-regional and seasonal variability in physical factors. In this study, mussels were collected from reference sites in Kiel (Germany) and Tvärminne (Finland) in three different seasons: summer and autumn 2016, and spring 2017. At biochemical level catalase, glutathione-S-transferase activities and lipid peroxidation in digestive gland and acetylcholinesterase activity in gills were measured. At cellular level changes in lysosomal membrane stability, lysosomal structural changes, lipofuscin and neutral lipid accumulation were selected. Changes in cell type composition in digestive alveoli, alterations in the digestive gland and changes in adipogranular cell density were examined to determine the responses at tissue-level. Histopathological alterations were examined in digestive gland, gonad and gills. Gamete developmental index was also determined. To characterize the ecological situation that was conditioning the biomarkers measured, analysis of the chemical tissue burden was performed and Chlorophyll-a and particulate organic carbon concentration and temperature changes were analyzed in each sampling locality using satellite remote sensing. In addition, an integrated biological responses index was performed to summarize the biomarker responses of each locality and season. Biochemical endpoints showed higher seasonal variability conditioned by temperature, food supply and reproductive cycle, while among the cellular endpoints, lipofuscin accumulation and lysosomal structural changes showed some kind of seasonal variation. Regarding tissue level biomarkers, only in Tvärminne seasonal changes were found, which were dictated by the more demanding energetic trade-off caused by reproduction. Histopathological analysis revealed differences between both sampling sites. It can be concluded that the ecological variables in each sampling site and season influenced the measured biomarkers in different ways, thus, to perform a correct assessment of the biological effect of a hypothetical pollution scenario in the Baltic Sea, biomarker baseline values and their response to environmental conditions must investigated in different localities and seasons. *Acknowledgement* - Funded by EU GRACE Project (nº 679266), CGL2016-76561-R (MINECO). UPV/EHU (UFI 11/37 fellowship, GIU 17/002) and Basque Government (IT810-13).

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The effects of the Deep Water Horizon oil spill on marine microalgae - A comparison of species sensitivity
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Limited algal toxicity data have been generated with crude or weathered oils as part of oil spill response and environmental impact assessment activities. Smithers Viscient conducted a series of toxicity tests using four standard microalgae species: *Dunaliella tertiolecta*, *Skeletonema costatum*, *Isochrysis galbana*, and *Thalassiosira pseudonana*, and one macroalga: *Ectocarpus siliculosus*. Eight toxicity tests for *Dunaliella* and *Skeletonema*, and four each for *Isochrysis*, *Thalassiosira*, and *Ectocarpus* were conducted with field-collected source and weathered oil samples collected during the Deepwater Horizon (DWH) MC252 Spill of U.S. National Significance. The subset of the data was evaluated to compare the species sensitivity of two of the algae tested, *Skeletonema costatum* and *Isochrysis galbana* for one of the weathered oil samples collected from the Gulf of Mexico during the DWH oil spill event. As crude oils are mixtures of poorly water-soluble hydrocarbons, exposures of weathered oil water-accommodated fractions (WAFs) were conducted at nominal loading concentrations of 100, 50, 25, 13, and 6.3% of a 1000 mg/L or 1.0 mL/L(oil) solution. Exposure solution samples were collected at the beginning and end of each exposure period for detailed chemical analysis of oil composition: volatile organic compounds (VOCs), parent and alkylated polycyclic aromatic hydrocarbons (PAHs), saturated hydrocarbon compounds (SHCs), and total petroleum hydrocarbons (TPH) or total extractable materials (TEM) concentrations. Oil toxicity was determined based on average specific growth rates and No-Observed Effect Concentration (NOEC) values. Results were also expressed based on the nominal loading percent WAF based on the dissolved sum of toxic unit (ΣTUs) of the whole oil. The data were compared to evaluate if the Target Lipid Model (TLM) is protective of algae based on the data for these species.

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Poster spotlight

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Achieving Social Equity, Protection of Ecology Systems, and Sustainable Economics

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The iCASS Platform: Nine principles for landscape conservation design
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The Anthropocene presents society with a super wicked problem comprised of multiple contingent and conflicting issues driven by a complex array of change agents. Super wicked problems cannot be addressed using siloed decision-making approaches developed by hierarchical institutions using science that is compartmentalized by discipline, nor can they be managed under the assumption that environmental conditions are stable. Adaptive solutions rest on human ingenuity that fosters transformation towards sustainability. To successfully achieve these objectives, conservation and natural resource practitioners need a paradigm that transcends single-institution interests and decision-making processes. Landscape ecologists link spatial patterns and processes to yield astute conservation recommendations. In practice, however, many of the recommendations that are informed by interdisciplinary science are not implemented. How can sustainability practitioners overcome this wicked problem? An oft-cited solution is to situate landscape ecology findings within a broader framework designed for achieving implementation. Increasing the knowledge base around this strategy is a current area of conservation innovation. We will present an innovation systems framework for an emerging and evolutionary step change in integrating landscape ecology into sustainability planning: landscape conservation design (LCD). We use existing governance and adaptation planning principles to develop an iterative, flexible framework—the "iCASS Platform." It consists of nine principles within five primary attributes—**i**nnovation, **c**onvening stakeholders, **a**ssessing current and plausible future landscape conditions, **s**patial design, and **s**trategy design. The iCASS Platform can facilitate LCD via processes that aim to create and empower social networks, foster stakeholder involvement, engender co-production and cross-pollination of knowledge, and provide multiple opportunities for deliberation, transparency, and collaborative decision-making. Our intention is to inspire a pivot from single-institution, siloed assessment and planning to stakeholder-driven, participatory design, leading to collaborative decision-making and extensive landscape conservation.

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The experience of two European projects on how to combine social, environmental, and economic values in mining
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Mining is usually a multifaceted topic due to complex and sometimes conflicting relations between different parties involved in the value ecosystem. The present work reports on how the challenges related to social, environmental, and economic sustainability in mining have been addressed by two European projects, namely ITERAMS, funded by the European Union H2020 programme, and SERENE, funded by the European Institute of Innovation and Technology. The purpose of this work is to explore the approach (and related challenges) aimed at identifying the different values generated by the mining projects and how these values may be combined and achieved both with the reduction of negative issues and risks and with the generation of positive impacts. The approach is based on mapping the stakeholders affected by the novel mining solutions foreseen by ITERAMS and SERENE, investigating the values potentially generated by the two projects for the different sustainability dimensions, and defining the related pressures and risks. Different tools and methods are used to perform the described approach, including literature research, a qualitative causal loop modelling, a social analysis, interviews with key stakeholders, and a preliminary Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) screening, followed by a full LCA, LCC and risk assessment evaluation. According to the focus of the projects, several water related values can be highlighted, such as water quality, availability, and management together with land preservation, health and safety, trust, respect of the regulatory standards, and monitoring of the system. The topics first identified at a general level are then characterized for the project validation sites in Finland, Portugal, and South Africa. This characterization is based on the definition of background situations which can affect the values, risks, and pressures. It is worth noticing that the meaning of the values identified may be different from one location to another because the risks and pressures which interact with these values are strictly dependent on the local environmental, social, socio-economic, cultural, and political context. The present contribution moves in the direction where balancing social, environmental, and economic issues does not mean that impacts can be shifted or compensated by another sustainability dimension, but rather the performance should be equally good and acceptable for all the stakeholders and topics involved.

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Precautionary risk assessment scheme for deep-sea mining

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The land-based deposits are depleting and the demand for metals is still increasing, giving rise to a growing interest in the exploration of mineral resources from the deep-sea. The International Seabed Authority has already given out 29 exploration contracts, whether for polymetallic nodules, cobalt-rich crusts or seafloor massive sulfides, and although until now the main focus was the exploration of the deep, the exploitation may soon begin. However, these resources cannot be considered in isolation since they are part of unique and most of the cases poorly understood ecosystems, such as hydrothermal vents. This has raised several concerns by the scientific community due to the possible, and probably irrecoverable, environmental impacts of the mining operations both, at the seafloor and along the water column till the surface, caused by the formation and dispersion of plumes, the movement of heavy machinery and the continues exposure to noise and light, in an environment where life evolved in the darkness. Such impacts will include within other aspects the loss of biodiversity, changes on organic matter degradation and nutrients cycling (nitrogen, phosphorus, silica, sulphur, and hydrogen), with subsequent loss of important ecosystem services. Taking this into account, lessons have to learned from the impacts on the mining activity on land, and precautionary measures should be taken to minimize negative environmental impacts before the exploitation phase.Hence, based upon currently available information and the precautionary principle approach we aim to propose a preliminary risk assessment framework to be applied during the prospection phase to support decision making before explotation contracts are granted.

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Challenges for the pesticide regulation in the EU: A German perspective

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Pesticide regulation of plant protection products (PPP) is an increasing complex process in Europe. Demands on the application are laid down in the legal framework and several guidance documents. However country specific approaches in risk assessment and risk mitigation increase the complexity of the authorization process. In some areas this complexity has the potential to result in a lack of harmonization for both risk assessment and risk management. Furthermore technical developments, state of science and technology in risk assessment and risk management, active substances with new mode of action such as RNAi based PPP, societal and political demands on agriculture production systems and as a consequence operationalisation of protection goals additionally challenge applicants, risk assessors, risk managers and decision makers. The presentation will highlight some of the most relevant challenges for the authorization of plant protection products from the perspective of the German competent authority. The current situation and critical aspects will be described and identified. For some aspects some first thoughts and ideas will be presented which might show the way forward to improve the authorization process of pesticide regulation.

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Protection goals for non-target terrestrial plants: Can precision application technology help ?

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EFSA's Scientific Opinion addressing the state of the science on the risk assessment of plant protection products for non-target terrestrial plants (NTTPs) was published in 2014. The Opinion defines non-target plants as all plants growing outside fields and those growing within fields that are not the intended pesticide target. It also states that non-crop plants growing in in-field areas provide ecosystem services including food web support, aesthetic value, genetic resources and endangered species, which require protection from the adverse effects of plant protection products. Since 2014, options for protecting ecosystem services provided by non-crop plants have been discussed in various stakeholder workshops. However, the regulatory debates around these issues rarely consider advances in agricultural technology (i.e. precision agriculture techniques) that have the potential to modify the way in which herbicides are applied in the future and will minimize the risks perceived to be associated with their use. Increasing precision enablement of crop sprayers includes the use of GPS systems, boom section control and pressure variation, so enabling variable applications across fields. Meanwhile, increased understanding of the environmental factors driving target weed distributions allows development of field-specific weed maps. Combination of these approaches will enable patch spraying whereby only the infested area of a field is treated. Estimates suggest that these approaches will permit a 6 to 81% reduction in the use of herbicides selective for broad-leaved weeds and a 20-79% reduction for products selective for grass weeds while use of automatic boom section control has been found to reduce use by 15.2 to 17.5% when compared with no boom section control[(1)]. This presentation aims to raise awareness of the potential environmental benefits of the use of precision technologies for weed control. If European agriculture is to benefit from these innovations in the future, the EU needs to retain access (i.e. registrations) to the necessary herbicide products by developing regulatory risk assessment processes that accommodate the use of these technologies. [(1)]Precision agriculture and the future of farming in Europe, Scientific Foresight Study (IP/G/STOA/FWC/2013-1/Lot 7/SC5), European Parlimentary Research Service Scientific Foresight Unit December 2016

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Food security and functioning ecosystems: the role of ecosystem services in framing protection goals

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Ecosystems and the biodiversity they support are essential to human survival, health and well-being. Ecosystem services are provided by landscape elements and hence an ecosystem services approach to environmental protection is inherently linked to how we manage landscapes. Half the habitable land in the world is managed by farmers and continuing growth in the human population will mean that the global demand for food will increase for at least another 40 years. People need both food security and functioning ecosystems. We therefore need to manage landscapes to optimise food production and other ecosystem services. However, because of trade-offs between yield, agricultural area and biodiversity, we can't have everything, everywhere, all of the time. An ecosystem services approach is one way to frame protection goals and help identify some of the trade-offs to be factored into land management decisions, including the use of chemicals. An ecosystem, services approach could result in better informed risk management decisions and more relevant environmental risk assessment by focusing protection goals on what stakeholders value. It increases transparency in trade-offs and prioritisation of ecosystem services (i.e. what to protect where and why) and offers the potential of a more holistic approach to environmental management. Ecosystems have the potential to provide many ecosystem services and adopting an ecosystem services approach requires greater ecological understanding, which may be a challenge for implementation. There is an urgent need to develop tools that either measure ecosystem services directly or produce information that can be robustly extrapolated to ecosystem service performance. Crop production systems are part of the wider landscape and are influenced by social, economic and policy drivers. Any reduction in the amount or type of food produced in Europe may result in unintended consequences on global biodiversity. This is because displacing food production also displaces environmental impact, often to areas with greater biodiversity value and weaker environmental controls than in Europe. We therefore need to take a holistic, systems-based approach to managing food production for the benefit of people and nature.

Human Health and Environmental Risk Assessment of Chemical Mixtures: Moving Towards the Non-toxic Environment (II)

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From words to action - How can chemicals legislation be improved to deal with groups of chemicals and take cumulative exposures and mixture toxicity into account?

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The need to develop regulatory strategies for assessing cumulative exposures and mixture toxicity has been pointed out in several policy documents on the EU level and in Sweden. Currently the pesticide and biocide legislations specifically require a cumulative risk assessment, and guidance for such assessments are available. However, regulatory approaches need to be developed also for other types of chemicals. Addressing mixtures increases the complexity of risk assessment and data demands, and grouping of chemicals might help structuring this complexity. Grouping might also facilitate read-across and help avoiding "bad substitution". To explore possible ways forward, the Swedish Government established a special inquiry. Here this inquiry will present its draft recommendations: 1. Single substances are regulated under different legislations and no agency or other authoritative body has the mandate to add up the exposures for the purpose of risk assessment. 2. The current risk assessment paradigm assumes that individual substances are released into an otherwise pristine environment. 3. There is no EU legislation that has the general scope and objective to ensure a good health status of humans, taking cumulative exposures to chemicals and mixture toxicity into account. 4. The effectiveness of chemicals risk management is undermined when a chemical with undesirable properties can be substituted with a chemical that has similar properties. 5. Monitoring can show unacceptable concentrations of chemicals in the environment or humans, but sufficiently effective feed-back loops to the rules controlling emissions are missing. 6. We lack information on real-life exposure patterns to mixtures, how exposures differ in time and space, and which chemicals drive mixture risks. 7. Authorities need resources to develop regulatory processes and practices that can make the implementation of chemicals legislation more efficient and up to date with current knowledge. For each of these issues an inventory of possible regulatory actions was compiled. The inventory includes proposals from the scientific literature and agency reports as well as input received during a number of stakeholder meetings. The resulting collection of proposals was combined with an analysis of the relevant EU legislations to identify concrete opportunities for improvement. The final recommendations will be presented to the Swedish Government by the 29th of September 2019.

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Is mixture toxicity behaviour a devaluated tool in the formulation of Plant Protection Products meeting new regulatory requirements?
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The production of eco-friendlier Plant Protection Products (PPPs) is a technical challenge and a regulatory requirement. The agrochemical industry has invested mainly in the reformulation of available PPPs to fulfill the regulation. However, during the developing stages of new formulations, the mixing of the ingredients has not been systematically tested, in order to identify effective combinations which may be less harmful to the environment. In this study, a commercial herbicide combining two active substances (a.s.), nicosulfuron and terbuthylazine, was used as model PPP. The a.s. were combined at different ratios to identify alternative formulations with reduced impacts in a non-target indicator (*Lemna minor*) representing potentially contaminated edge-of-field surface waters. Single and mixture toxicity tests with *L. minor* revealed that these a.s. act antagonistically towards the macrophyte, when combined. This suggests a protective effect of the combination compared to the a.s. dosed individually. The a.s. were also tested for efficacy, both singly and in mixture, towards a target weed for this commercial formulation, the weed *Portulaca Oleracea.* Likewise, the a.s. revealed an antagonistic behavior throughout the whole response surface. The study showed that (i) there were no environmentally safe a.s. combinations ensuring target-efficacy; (ii) terbuthylazine alone was effective in controlling *P. oleracea* with no environmental hazardous potential, dosed at concentrations 10-fold lower than those involved in commercially recommended application doses. Overall, this case-study suggests that modelling tools widely used in the field of environmental risk assessment of PPPs may also be useful in PPP design stages for a more efficient joint meeting of efficacy and environmental friendliness requirements.

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Aquatic community responses as consequence of a realistic multiple low dose pesticide exposure scenario in stream mesocosms
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Usual agricultural practice is the application of a set of different types of plant protection products (PPPs) within a crop-specific spraying sequence during a season. Due to discharges aquatic communities inhabiting adjacent streams are consecutively exposed to a set of pesticides, which may cause a multiple-stress for non-target organisms. However, potential additive or even synergistic effects of realistic exposure scenarios are rarely investigated, especially on the community level. Here, we studied the effects of a pesticide spray sequence for apple cultivation on aquatic communities in surface waters using eight indoor stream mesocosms. Donated concentrations of the active substances were based on the specific regulatory acceptable concentrations (RAC) derived from the risk assessment of each PPP, a specific concentration considered as acceptable to occur in surface waters. We hypothesize that the exposition by a realistic spray sequence of pesticides using product-specific RACs has no effect on aquatic communities in stream mesocosms. Overall, nine fungicides, four insecticides and four herbicides at nineteen days were applied at their respective RACs for surface waters to each of the four treatment mesocosms within a period of more than 100 days. By lack of serious effects, a set of pronounced effects were monitored on structural and functional levels: benthic invertebrates and emerging merolimnic insects showed an effect on the community level at the end of the experiment indicating an effect of the multiple stress scenario event at the applied low concentration level (RAC). Furthermore, several single taxa showed a significant higher variability in the treatment mesocosms. Congruently, a reduced primary production in the macrophyte *Potamogeton natans* and a slower decrease in the potassium concentration in the water phase were measured over time indicating a bias between primary production and respiration processes at the community level. This study indicates the need to consider realistic multiple stress induced by PPP in crop-specific spraying sequence for the evaluation of potential effects on non-target organisms also on the long term and partly beside classical endpoints for mesocosm experiment. Thus it is still necessary to consider carefully if the overall level of protection intended by the legislative framwork (1107/2009/EC) are reached by the current practice of environmental risk assessment, i.e. for individual PPPs separately.

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TBD

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From individuals to populations: evaluation of laboratory-based predictions for toxicity of neonicotinoids and their mixtures to sensitive aquatic insects
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Widely applied due to their favourable physicochemical characteristics (e.g. persistence, hydrophilicity), broad pest spectra, and versatility in use, neonicotinoids have become ubiquitous freshwater contaminants. Rather than single compounds, neonicotinoids are likely to be detected as mixtures, posing a risk to aquatic insects inhabiting contaminated environments. Recent laboratory studies have shown that neonicotinoid mixtures can elicit greater-than-additive toxicity in the aquatic insect, *Chironomus dilutus*. However, this has yet to be validated under field conditions. In this study, we characterized the chronic (28 and 56 d) toxicity of three compounds (imidacloprid (IMI), clothianidin (CLO), and thiamethoxam (TMX)) and their mixtures to natural insect communities. Using experimental *in situ* enclosures (limnocorrals), we exposed wetland insects to neonicotinoids/mixtures at theoretically equitoxic concentrations (1 toxic unit, Concentration Addition) and evaluated their toxicological effects on both the composition of all emerged insect taxa and cumulative Chironomidae emergence over time. Neonicotinoid treatment subtly shifted community composition, significantly increasing the relative proportions of emergent Trichoptera and Odonata in treated enclosures. Cumulative emergence increased over time in all limnocorrals, and there was a significant interaction between time and treatment. At 28 d (study midpoint), cumulative Chironomidae emergence was not significantly different between neonicotinoid treatments and controls. However, cumulative emergences in IMI, CLO, and CLO-TMX treatments were 42%, 20%, and 44% lower-than-predicted from applied doses. At 56 days (study cessation), effects on cumulative emergence became significant for these three treatments. However, due in part to high intra-treatment variation, mixtures were not more toxic than single compounds (after 28 or 56 d). Therefore, laboratory-derived predictions did not adequately account for neonicotinoid/mixture toxicity under field conditions. Observed greater-than-predicted single compound and concentration additive mixture toxicity should be accounted for in neonicotinoid-based environmental risk assessments and regulatory actions. This study indicates that single-species tests may not adequately predict the effects of neonicotinoids on sensitive aquatic insect populations, and, most importantly, demonstrates the need for field-validation of laboratory-derived toxicity predictions.

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Poster spotlight

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The Environment as a Reactor Determining Fate and Toxicity of Nanomaterials (IV)

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Exploring the Nano-Cosm: Advances in multi-element single-particle Analysis for Nanoparticle Characterization in the Environment
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The advances in engineered nanotechnology lead to a commensurate development of sophisticated and sensitive analytical techniques capable of detecting and characterizing nanomaterials in complex environmental matrices. Though early single particle ICP-MS (spICP-MS) has demonstrated considerable promise in providing the size and number concentration of nanoparticles in very simple combinations of nanoparticles and environmental systems, it has been limited through its inability to accurately quantify more than a single element per particle, lacking compositional and identity information. The introduction of single-particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOF-MS) enables multi-element quantitation on a particle-by-particle basis, opening the door for both more accurate assessment of engineered nanoparticle exposure, and the exploration of geochemical processes at the nanoscale. By virtue of time-of-flight, almost the entire atomic mass range (7-250 m/z+) can be determined in individual particles allowing for the identification and seperate quantification of natural and engineered nanoparticle populations. In general elemental and isotopic ratios enable to differentiate nanoparticle populations (natural, incidental and manufactured) and deliver information regarding chemical composition and structure. In this work the utility of spICP-TOF-MS is demonstrated in the analysis of natural waters and soil particles, exemplifying the potential of this technique to monitor nanoparticles and processes such as aggregation, accumulation and pollutant (co-)transport. Examination of elemental compositions

of particle constituting elements as aluminum, silicon, iron, and manganese provides the information on the composition of natural mineral nanoparticles and co-occurrence of other elements, such as lead and copper demonstrate potential transport of other contaminants. Colloid facilitated transport is further demonstrated in the analysis of soil particles extracted from a cadmium/antimony mine, where arsenic bound to iron-bearing minerals can be detected and quantified. The development of this technique provides an avenue to not only discriminate engineered nanoparticulate contaminants from natural nanoparticle populations but may also further develop our understanding of nanogeochemical processes in the environment.

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Suspended particulate matter (SPM): The known unknown in nanomaterial risk assessment

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In light of regulations' requiring environmental fate assessment of engineered nanomaterials (ENMs), a major distinctive feature of ENMs – their particulate nature – precludes direct application of testing protocols or fate models designed for dissolved substances. In the aquatic environment particle-particle interactions (influencing ENM transport, behaviour and uptake) need to be considered. These include homoaggregation among ENMs and heteroaggregation with natural suspended particulate matter (SPM). From an ENM particle's perspective, SPM surfaces are omnipresent, as size and most likely number concentrations exceed those expected for ENMs. Thus, heteroaggregation is assumed to be the dominant process. Covering heteroaggregation in fate assessment requires a thorough scientific process understanding and informed simplifications of the same, feeding into the design of controlled experiments that allow deriving fate parameters. The most blatant "known unknown" is the interaction of ENMs and natural SPM. To fill this gap, process-relevant, near-natural SPM analogues are needed: natural SPM is very complex, featuring flock-like structures composed of various minerals and organics from the molecular level to microorganisms or detritus. Compositions, concentrations and sizes are dynamic in space and time. Hence, a mere mineral analogue is too simplistic, while attempts to rebuild natural SPM from major mineral fractions identified did not arrive at similar flocks. Reviewing available literature on natural SPM, we identified the heterogeneity of physicochemical surface characteristics as a crucial feature and took a conceptual approach, selecting mineral and organic components based on the interplay of abundance, physicochemical heterogeneity and realistic mass ratios. Here we present our resulting SPM analogues: complex flocks consisting of illite, hematite, quartz and tryptophan (proxy for microbial exudates), representing heterogeneity of natural SPM. They are reproducible and stable in batches, but at the same time dynamic when varying hydrochemistry – reflecting natural processes of flock association/dissociation. For the first time, such near-natural analogues were employed in heteroaggregation studies with ENMs, allowing more environmentally relevant conclusions for ENM fate assessment and modelling. However, the scope may also be extended to other particulate contaminants like micro-/nanoplastics, soot or tire wear – and perhaps beyond...

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Predicting nanomaterial fate in the environment using a spatiotemporal multimedia model: An application to the River Thames, UK

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The increasing use of engineered nanomaterials (ENMs) in modern society means an increased risk of release to the environment, either during their production, use or at end-of-life. It is therefore crucial that we are able to assess where and in what form ENMs end up in the environment. However, the particulate nature of ENMs and the fact that their transformations are driven by kinetic rather than equilibrium processes necessitates the creation of nano-specific tools to enable such assessment. Modelling plays a key role in predicting ENM fate, allowing us to fill gaps where measurement data are not available and to explore theoretical scenarios. Recently, there has been significant progress to this end, but models published to date have been limited in spatial or temporal resolution, and often consider only one or two environmental compartments. Work within the Horizon 2020 project NanoFASE (Nanomaterial Fate And Speciation in the Environment) aims to address this. The NanoFASE model, under development as part of this project, will be the most advanced spatiotemporal multimedia model of ENM fate, speciation and biouptake to date. The model represents a catchment by subdividing it into a grid of regular size, each grid cell containing one or more environmental compartments – in this example, soils, freshwaters and their bed sediments. Each compartment acts as a separate reactor for transport and fate processes, including percolation of water through soil, erosion of topsoil into surface waters, bioturbation of soils, river discharge, sediment deposition/resuspension, and ENM–specific processes such as heteroaggregation with river sediments, dissolution and attachment (i.e. to the soil matrix). Here, we present the model as applied to the Thames catchment in the UK, in doing so highlighting the importance of a number of nano-specific and non-nano processes to NM fate, such as bioturbation of soils, attachment to the soil matrix and deposition/resuspension to/from bed sediment. The NanoFASE model will be further developed for ENMs with more complex transformations such as dissolution and chemical transformation, and kinetic models for uptake by biota will be incorporated. Model outputs will be used to help validate lower-level screening models, such as SimpleBox4.0-nano, relevant for chemicals regulation.

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Assessing dissolution of silica nanoparticles in aqueous media

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While the application range of SiO_2 nanoparticles (SiO_2-NPs) is broadening, many of the current and new uses can increase their accumulation in the environment. First predictions for engineered SiO_2-NP concentrations in environmental surface water are 0.12 μg/L, and the mean concentration in soil is estimated to be 0.43 μg/L [1]. Therefore, it is crucial to gain more information about the degradation of SiO_2-NPs in the environment, which is currently scarce. Here, we present the results of a method development that was used to quantify the dissolution rate of SiO_2-NPs in aqueous media. Silica was quantified using inductively coupled plasma optical emission spectroscopy (ICP-OES) and a colorimetric assay. After initial tests of a variety of classic dialysis membrane systems, we adopted the membrane-free setup presented in Figure 1A. This allows direct measurements of the unaltered dissolution rate. To validate this setup, we quantified the dissolution SiO_2-NPs at pH 11 over 72 h in parallel with a membrane-free and a membrane setup. The high pH 11 was chosen because of the known fast base-catalyzed hydrolytic degradation of SiO_2 in this pH range [2]. Using an initial concentration of 300 mg SiO_2/L, complete dissolution was observed within the first 50 hours in the membrane-free setup. The data was fitted to a *pseudo*-first order kinetics model $d(t) = d_{max}(1-e^{-kt})$, where d is the percentage of dissolved Si. This allows estimating a half-life of about 10.2 hours for SiO_2-NPs in Milli-Q at pH 11. The particle dissolution was confirmed by the TEM micrograph. We currently use this system to evaluate particle dissolution at environmentally relevant pHs. Membrane-free dissolution setups are a versatile tool to produce data for the fate and behavior of SiO_2-NPs in aqueous media which is important for the regulation of this nanomaterial. [1] Wang Y, Kalinina A, Sun T, Nowack B. 2016. Probabilistic modeling of the flows and environmental risks of nano-silica. Sci Total Environ 545-546:67-76. [2] Croissant JG, Fatieiev Y, Khashab NM. 2017. Degradability and clearance of silicon, organosilica, silsesquioxane, silica mixed oxide, and mesoporous silica nanoparticles. Advanced Materials 29:1604634. *Acknowledgement* - The authors thank the Swiss National Science Foundation (project 168187) and the Adolphe Merkle Foundation for the support and funding of the study.

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Formation and transformation of carbon nanoparticles from environmental sources

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The last decade of environmental carbon nano-science has often focused on understanding the behavior of well-defined engineered carbon nanoparticles (eCNPs). However, in the natural environment more complex and heterogeneous carbon nanoparticles deriving from pyrogenic and petrogenic sources (pCNPs) are more than four orders of magnitude more abundant. The sources of pCNPs include fossil fuel combustion, forest fires, fossil coal, activated carbon and biochar application. A key aim of this presentation is to compare and transfer findings from studies on eCNPs to the fate and transformation of the more heterogeneous pCNPs. Findings from previous investigations into the chemical, physical, and biological transformations of larger heterogeneous carbonaceous particles, as well as of eCNPs, can help us to understand the transformation of pCNPs. To bridge findings on colloidal behavior, particulate transport and interactions with NOM from eCNPs to pCNPs a literature review was recently published (doi: 10.1039/c8en00676h). Based on the knowledge gaps identified, we designed a first study on soot particle transformation and colloidal behavior. We review key processes and factors important for pCNP transformation and discuss results from an experimental study with soot. Based on our findings, future research on pCNPs should further investigate pCNP formation and their transformation in natural environments, specifically focusing on changes in porosity, and on interactions with non-carbonized, tar, and mineral phases, which have commonly not played important roles for eCNP research.

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Influence of weathered multi-walled carbon nanotubes on the distribution of the biocide triclocarban in a sediment-water system
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Multi-walled carbon nanotubes (MWCNT) are widely used nanomaterials in a variety of different products and processes, and are well-known for their high sorption capacity. Due to increasing usage and production, exposure to the aquatic environment, either accidentally or via disposal of CNT-containing products, might increase likewise. Weathering processes like sunlight radiation can alter nanoparticle properties and lead to changes in their environmental behaviour. The retention time of MWCNT in the water phase is very short; due to agglomeration and aggregation processes. They preferably settle down to sediments, which act as sink for CNT and other pollutants. During their residence time in the water phase they may interact with water dissolved xenobiotics, like triclocarban (TCC) and alter the fate of these substances. In the present study MWCNT were irradiated by simulated sunlight for 90 d. These weathered MWCNT (wMWCNT) were used to investigate their influence on fate and distribution of the biocide TCC in a sediment-water system. TCC was chosen because of its hydrophobic properties and strong affinity to sorb on MWCNT. ^{14}C-TCC and wMWCNT were shaken for 2 h before application to the system to ensure complete adsorption (loading process). Samples were incubated after application for 181 d in the dark at 19 °C (weekly aeration). Work up of samples was performed according to OECD Guideline 308. Residence time of radioactivity in the water phase was significantly prolonged in the presence of wMWCNT. In presence and absence of wMWCNT DT_{50} values for dissipation from the water phase of 0.42 d and 0.25 d, respectively, were calculated. Sedimentation time of ^{14}C-wMWCNT alone was assessed in a second study, using the same experimental set up. A DT_{50} of 3.21 d was calculated for the dissipation of ^{14}C-wMWCNT from the water surface. The amount of radioactivity found in extractable and non-extractable TCC residues from sediment was not altered significantly. An investigation of metabolites in methanol extracts using radio-thin layer chromatography could confirm the presence of metabolite 4-chloroaniline in one sample without wMWCNT. Presence of ^{14}C-TCC was confirmed in all tested samples. Mineralisation of ^{14}C-TCC was significantly reduced in presence of wMWCNT. Acknowledgements: The work is supported by the European Project NANO-Transfer that receives funding from the Bundesministerium für Bildung und Forschung (BMBF) under agreement with the FP7 ERA-NET SIINN.

Examining Behavioural Effects of Chemical Contaminants and Other Stressors on Behaviour, Ecology and Evolution of Wildlife

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The effect of two pesticides on the olfactory-mediated antipredator response of zebrafish (Danio rerio)
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Escaping predation is essential for the survival of any organism. Fish are warned about the presence of predators via an antipredator substance that is released from the skin of an injured conspecific. This substance is perceived by the olfactory system and initiates an antipredator response that is specific for the respective fish species. If an individual fails to respond to such an antipredator cue, chances of survival decrease substantially. The aim of the present study was to assess the effects of two pesticides on the antipredator response of zebrafish. Furthermore, an analysis script for the automated identification of certain behaviors from tracking data should be developed to accelerate the evaluation process. To this end, zebrafish were exposed to 0.01 % DMSO (solvent control), 2, 5 and 20 μg/L chlorpyrifos or 10, 100 and 300 μg/L linuron for 24 hours. Hereinafter, behavioral trials were conducted and videotaped. Following twenty minutes of acclimatization, either water (blank stimulus) or skin extract (alarm cue) was delivered and fish behavior was recorded for another ten minutes. Subsequently, video recordings were analyzed using EthoVision XT 11.5 (Noldus, Netherlands) and tracking data was was further analyzed using R (R Core Team) to automatically identify the behaviors of interest – namely bottom-dwelling, freezing and erratic movements. Following the delivery of skin extract, significantly more zebrafish exposed to 0.01 % DMSO exhibited bottom-dwelling and freezing behavior compared to following the delivery of water. Exposure to 100 μg/L linuron resulted in a decreased number of fish showing bottom-dwelling behavior compared to the skin extract solvent control, however, in the highest test concentration, this tendency was not visible anymore. Chlorpyrifos significantly reduced the number of bottom-dwelling fish in a concentration-dependent manner. Whereas the exposure to linuron did not affect freezing behavior, following exposure to chlorpyrifos fish froze significantly less. In conclusion, we showed that the antipredator behavior of zebrafish was significantly impaired following exposure to chlorpyrifos. As this behavior is essential for the survival of fish, its impairment could have fatal consequences for the individual. In addition, we developed a script for the automated analysis of the antipredator behavior of zebrafish, which greatly simplified and accelerated data evaluation.

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Determining the effects of psychotropic drugs in a marine and freshwater amphipod
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The use of behaviour in ecotoxicology is increasing, providing a link between biochemical and ecological effects of environmental contaminants. Psychotropic drugs are designed to modulate behaviours in humans, and preclinical studies have demonstrated that these compounds can alter behaviours in aquatic vertebrates. The effects of behavioural modulating drugs have been tested from a pharmacological discipline using anxiety-like behaviours including thigmotaxis and scototaxis on mice and zebrafish, using well-defined behavioural assays. These methods have been translated to ecotoxicological studies on vertebrates but comparatively few have been done on invertebrate species. This ongoing study aims to translate these techniques to model crustaceans for the purpose of high-throughput assessment of environmental risk, using both antidepressant and anti-anxiety compounds with different modes of action. Specimens of the marine amphipod, *Echinogammarus marinus* and the freshwater amphipod *Gammarus pulex* were exposed to the SSRI fluoxetine, or the BZD Oxazepam at a range of concentrations (0.001-1 µg/L). Swimming behaviours, as well as thigmotaxis and phototaxis were analysed using custom choice chambers in a DanioVision observation chamber with EthoVision software after 1 day, 1 week, and 2 weeks of exposure. Behaviours were observed under a light-dark cycle with 'light on' used as a stimulus to initiate behaviours. Both *E. marinus* and *G. pulex* showed alterations in swimming activity between light and dark cycles. Differences in velocity were also observed with duration of exposure to both compounds in *E. marinus* but not between treatments. In choice experiments, both species showed negative phototaxis and positive thigmotaxis during light cycles. Significant differences were observed in choice behaviours between treatments and with length of exposure (p=< 0.05) for Fluoxetine but not with Oxazepam. Results indicate that behavioural modifying compounds can have significant effects on velocity and phototactic behaviour. This ongoing study is further trying to ascertain whether compounds with differing modes of action can have different effects on amphipod behaviours, and whether seemingly similar species exhibit differences in their sensitivity to behavioural modifying compounds. We discuss our results in relation to inter- and intra- species variability highlighting the need to go beyond the mean, consider phenotypic plasticity and the spread of data.

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Impact of the two antidepressants citalopram and venlafaxine on brown trout (Salmo trutta f. fario) and great ramshorn snail (Planorbarius corneus)
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Citalopram (CIT) and venlafaxine (VEN) are the most frequently prescribed antidepressant drugs in Germany. CIT belongs to the SSRIs (selective serotonin reuptake inhibitors) and VEN to the SSNRIs (selective serotonin and noradrenalin reuptake inhibitors). Due to high consumption rates and poor elimination during wastewater treatment both antidepressants can be found in concentrations up to 1 μg/L in surface waters. Since up to now, consequences of the exposure to these pharmaceuticals for aquatic wildlife are far from being understood, we investigated responses of brown trout and great ramshorn at different biological levels. Brown trout eggs were chronically exposed in the eyed-ova stage to 0, 1, 10, 100, 1000 μg/L CIT and VEN in a semi-static three block design at 7 and 11°C for 5 months. Juvenile fish and snails were exposed in a similar design for 4 weeks at 7 respectively 11 °C. As apical endpoints, mortality, weight and length, and, for brown trout larvae only, hatching rate and heart rate were recorded. During the experiments, behaviour of fish was quantitatively assessed. Also water concentrations of citalopram were analysed and nominal concentrations complied with real concentrations. After exposure to 1000 μg/L CIT, weight of fish and snail, as well as length of fish were significantly reduced. In general, fish exposed to both antidepressants showed increased swimming activity and a predominant sojourn in the upper half of the aquaria. In addition, it became obvious, that in an artificial swimming measurement device (small aquaria of 17x17x8,5 cm), which had to be brightly illuminated to obtain optimal tracking results, brown trout larvae were highly active except for those exposed to 1000 μg/L CIT, which moved significantly less with a lower velocity than control fish under these stressful conditions. Snails exposed to VEN showed a significant increase in sole detachment. Mortality in fish and snails as well as hatching rate and heart rate of

brown trout larvae were not influenced by the two antidepressants tested so far. In general, the results of this study make evident that both antidepressants evolve their anxiolytic effects also in non-target organisms like brown trout or invertebrate species like the great ramshorn snail. The present study is part of the project Effect-Net which is funded by the Ministry of Science, Research and the Arts of the Land of Baden-Württemberg within the framework of the Water Research Network Baden-Württemberg.

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Antidepressants in the water: effects of field-realistic fluoxetine exposure on sociability and anxiety-related behaviour of fish
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Pharmaceutical pollution is a serious, global issue. Indeed, the rise in production and diversification of pharmaceuticals—and synthetic chemicals more generally—is equal to, or exceeds, most other recognised drivers of global change. Of particular concern are pharmaceutical pollutants with evolutionarily conserved drug targets and the ability to alter biological function at minute concentrations. The antidepressant fluoxetine is one such pollutant, which has the potential to disrupt behavioural pathways through impacts on the central nervous system and neuroendocrine system. Despite this, it remains unclear whether environmentally relevant fluoxetine exposure can adversely impact behavioural processes in wildlife. To address this knowledge gap, we investigated effects of two field-realistic concentrations of fluoxetine (x: 60.5 and 351.9 ng/L) on sociability and anxiety-related behaviours in male and female eastern mosquitofish (*Gambusia holbrooki*). Sociability and anxiety-related behaviours are known to regulate important inter- and intraspecific interactions and, if disrupted, have the potential to impact the fitness of exposed wildlife. Additionally, we measured whole-body tissue concentrations of fluoxetine and norfluoxetine (fluoxetine's primary metabolite). We found that fluoxetine exposure altered anxiety-related behaviour, whereas no significant effect of exposure on sociability was observed. What is more, observed impacts of fluoxetine on anxiety-related behaviour were sex-specific, with females showing reduced anxiety-related behaviour at the lower dosage, while males showed an increase at the higher dosage. Fluoxetine and norfluoxetine were present in the tissue of male and female fish in both the low (x: 5.5 and 10.1 ng/g, respectively) and high dosages (x: 10.3 and 26.8 ng/g, respectively). Further, the bioconcentration of fluoxetine and norfluoxetine was size-dependent, with smaller fish showing higher relative tissue concentrations of fluoxetine and norfluoxetine. In combination, these findings provide evidence that fluoxetine at field-detected levels can alter the behaviour of fish, but also suggest that the magnitude and direction of these effects are dependent on sex and dosage. Moreover, our results provide insights into the apparent disparity of previously reported behavioural effects resulting from fluoxetine exposures.

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Fish larve response to nanoplastics: Is behavior as a sensitive endpoint?
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The presence of plastics in the environment is a global issue. In the environment plastics can degrade into increasingly smaller particles reaching the nanometer size which increases their potential to be incorporated by organisms. However, the current understanding of the consequences of these particles can be considered still scarce. The knowledge of the effects of nanoplastics to aquatic organisms may be considered limited, particularly in terms of effects on behavior. Behavior, as the outcome of many developmental and physiological processes resultant from interactions between the organisms and the surrounding environment, has attracted the attention of ecotoxicologists as a sub-lethal endpoint with ecological relevance. If biochemical effects are translated into fitness-related traits such as decreased ability of respond to stimulus, escape from predators and pernicious environments, they can have a strong impact on the ecosystem functioning on a long term. This study aimed to assess if 50 nm polystyrene (PS) and polymethylmethacrylate (PMMA) nanoparticles could affect fish larvae behavior, after 96 h. Data showed that behavior endpoints were sensitive to exposure to nanoplastics emphasizing the potential pernicious effects of these particles in the environment.

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The Saturday night fever - effect of benzodiazepine cocktail on fish behavior and bioconcentration
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The aim of this study was to describe possible synergistic effects of pharmaceutically active compounds (PhACs) on fish behavior. Four benzodiazepines (oxazepam, temazepam, bromazepam, and clobazam) were chosen as an example of PhACs with similar mode of action that also frequently occur together in the environment. Juvenile European perch (*Perca fluviatilis*) was used as an indicator fish species to study behavioral changes induced by both exposure to single compounds and their mixtures. Fish were exposed to target PhACs at two concentration levels (8 and 0.5 μg L^{-1}), where the low dose is considered as environmentally relevant. Unique approach using a structured light sensor was used for automated tracking of fish in three-Dimension during the boldness, sociality, and activity trials. All fish were sacrificed after the experiment, muscle tissue was sampled, and levels of target PhACs were analyzed using the method of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The potential to modify fish behavior in this study strongly depended on the target compound, specific behavioral trait, and concentration level. However, clobazam expressed the highest potential to alter boldness, sociality, and activity of studied fish. No clear evidence of synergistic effects was found, as the exposure to benzodiazepines mixture mostly resulted in similar effect as exposure to the most potent individual compound in each behavior trial. Bioconcentration also differ greatly between the target compounds. Temazepam was found at the highest levels in fish muscle tissue, reaching the mean concentration of 52 ng g^{-1} after seven days of exposure to 8 μg L^{-1}. Five times lower mean concentration was found in case of bromazepam that was the compound with lowest bioconcentration potential. Relatively high concentrations of oxazepam were also measured in muscle of fish exposed to temazepam. This finding is most likely due to fish metabolic activity, as oxazepam is known as a transformation product of some other benzodiazepines. Except of oxazepam, where both waterborne exposure and production via metabolic activity occurred, all other target compounds express lower bioconcentration potential in mixture than in individual exposure.

Benefits of BiER: How Biotransformation and Elimination Rate Related Science Can Improve the Regulation and the Sustainable Use of Chemicals

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Making the IVIVE formalism fit for regulatory purposes
S. Krause, K. Goss, Helmholtz centre for environmental research - UFZ / Analytical Environmental Chemistry
The assessment of the bioaccumulation potential is one criterion in the evaluation of chemicals regarding their potential harmfulness for the environment. Among the processes influencing bioaccumulation of a chemical, elimination via biotransformation is the only one that cannot be estimated from physico-chemical or physiological information. At the same time, however, elimination via biotransformation can be the key parameter to reduce the internal concentration of a hydrophobic chemical and, by this, preventing bioaccumulation. Accordingly, biotransfomation has to be accounted for in bioaccumulation assessment and experimentally determined biotransformation rates are required. In line with the effords of reducing the use of animal testing, the method of choice for determination of biotransformation rates are in vitro experiments and subsequent extrapolation to the in vivo rate. Currently, there are often significant discrepancies between extrapolation results and in vivo observations reported. When evaluating reasons for the discrepancies between predictions of biotransformation kinetics from in vitro data and in vivo observations, we identified a couple of potentially relevant processes that are currently not considered in the extrapolation procedures: the potentially limited delivery of the chemicals in vivo due to slow permeation of the chemical into the metabolically active cells or due to slow desorption from binding plasma-proteins, the potentially decreased uptake of the chemical due to first-pass effects, the potentially higher biotransformation capacity of the liver due to the tubular architecture of blood capillaries or the potentially increased elimination due to extrahepatic metabolism. To estimate the relevance of the above mentioned processes under physiological conditions, we evaluated experimental data from the literature in suitable physiologically based modelling approaches. This evaluation helps to improve our understanding of the complexity that is required for reliable in vitro-in vivo-extrapolation and how the currently observed discrapncies between extrapolation results and in vivo observations could be solved. Furthermore, the generated insights are not only relevant for bioaccumulation assesments in fish, but provide also useful information in the emerging field of bioaccumulation assessment in terrestric organisms.

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Evaluation of major uncertainties as part of in vitro-in vivo extrapolation to predict the bioaccumulation potential of fragrance chemicals
H. Laue, Givaudan Schweiz AG / Fragrances S & T; L. Hostettler, Givaudan

Schweiz AG; K. Jenner, Givaudan / Global Regulatory Affairs & Product Safety; G. Sanders, Givaudan International SA / Regulatory Affairs and Product Safety; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology; A. Natsch, Givaudan Schweiz AG / Fragrances S &T

Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals which usually involves the determination of the bioconcentration factor (BCF) in fish. *In vitro* systems measuring *in vitro* intrinsic clearance rates of chemicals have been established as alternative methods to refine *in silico* BCF models which are based on hydrophobicity (i.e. log Kow) and were recently adopted as new OECD test guidelines (319A,B). *In vitro-in vivo* extrapolation (IVIVE) models can then be applied to estimate liver clearance and a "whole body" biotransformation rate constant (k_{MET}) to refine BCF predictions. Several published studies show a clear tendency for overprediction of predicted BCFs based on IVIVE. Errors in estimates of the fraction unbound (fu) and the impact of the *in vitro* test concentration were discussed as possible reasons. With the OECD approved TGs available, testing and optimizing the IVIVE models is now of high relevance. The goal of this study was to provide a large dataset on industrial relevant chemicals, mainly fragrance chemicals, with measured *in vivo* BCF data for which we determined biotransformation rates using liver S9 sub-cellular fractions from rainbow trout (RT-S9). BCFs were predicted with an IVIVE model parameterized with different assumptions to evaluate which of the postulated reasons could account for the commonly observed overprediction of BCFs. Fu was measured in RT-S9 and trout serum using a vial equilibration method and lower test concentrations were applied for a subset of 6 chemicals. The majority of the 22 fragrance chemicals was moderately to rapidly transformed in RT-S9, whereas 5 chemicals with measured BCFs >2000 L/kg were not biotransformed. A systematic overprediction of the BCFs was observed for the majority of the chemicals tested using the standard IVIVE model (fu calculated), whereas predicted BCFs were similar to *in vivo* BCFs when the correction factor fu was set to 1. The incorporation of measured fu values in the IVIVE model parameterization and lowering the test concentrations below 1 mM did not significantly improve the BCF predictions. This is the first significant dataset tested under the new OECD conditions. Neither testing below 1 μM, nor incorporation of measured fu in the IVIVE model avoided the commonly observed tendency to overpredict BCFs. This suggests that these experimental refinements cannot resolve the mispredictions and further improvements of IVIVE models are needed.

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A tiered testing strategy for rapid estimation of bioaccumulation by a combined modelling - in vitro testing approach: derivation of kinetic rate constants in different in vitro models

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Quantification of chemical toxicity continues to be based mainly on measurements of external exposure. Yet, in order to understand, interpret and extrapolate toxic effects, using internal concentrations of chemicals is more suitable. Though these can be measured, being able to predict them would be much more efficient and feasible. Predicting chemical internal concentrations accurately is, however, still challenging as biotransformation processes can strongly influence the extent to which chemicals accumulate in fish. Existing approaches thus far are based on the assumption that biotransformation in the body solely occurs in fish liver. Since this is unlikely to be true, this assumption may result in underestimating the biotransformation of chemicals in fish. Therefore, in our study, in addition to liver, we focus on rainbow trout gill and intestine as potential sites of chemical biotransformation. We have determined *in vitro*biotransformation rates for several chemicals in an array of models based on rainbow trout *(Oncorhynchus mykiss)*: a primary gill cell culture, S9 fractions of liver and intestine, as well as permanent cell lines from, respectively, gill (RTgill-W1), liver (RTL-W1) and intestine (RTgutGC). Except for 2,4,6-Tribromophenol (TBM), the biotransfomration of chemicals was the most significant in liver, followed by intestine, followed by much slower biotransformation observed in gills. The *in vitro*intrinsic clearance (CL_invitro) of TBM, determined with liver S9 fraction, was one order of magnitude lower than for other chemicals while it was in the same order of magnitude as for the other chemicals for the primary gill cell system. For TBM no biotransformation was observed in permanent cell lines. For all other chemicals, the respective CL_invitro were comparable across the different *in vitro*systems. Our study shows that chemical biotransformation takes place not only in liver but also in fish intestine and gills. Thus, accounting for their CL_invitro could lead to more accurate estimation of chemical accumulation and bioconcentration factors (BCF). In addition, while the biotransformation capability of permanent cell lines requires further investigation, they could potentially be applied as a first screen to confirm that a compound is biotransformed in fish. Finally, the new measured datasets for biotransformation in various biological compartments and tissues could provide quantitative information to support a Weight of Evidence approach.

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An attempt to account for biotransformation in bioaccumulation models for aquatic invertebrate species.

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Toxicokinetic (TK) models describe the process of bioaccumulation as a balance between contaminant uptake from different sources (e.g. water or diet), and their elimination by different processes (excretion, growth and/or biotransformation). TK model parameters are generally estimated by fitting the model to data from short-term exposures of organisms under controlled laboratory conditions. The model fit is often based on a deterministic approach, which cannot take simultaneously into account different types of data (e.g. bioaccumulation and growth data), nor account for correlations between parameters. In this context, the uncertainty related to model parameters, and thus to model predictions, is not correctly estimated. Moreover, in invertebrates, biotransformation is generally neglected, assuming a low metabolic activity. However, some species such as the amphipod *Gammarus pulex* are capable to metabolize a wide range of organic chemicals. The aim of our study is to incorporate biotransformation into a generic TK model for aquatic invertebrates, based on a unified Bayesian framework. We attempted to adapt one of the methods described in the literature for estimating biotransformation rate (k_m) values, so as to estimate simultaneously model parameters from uptake and elimination data. We illustrate this approach by fitting the generic model with some data available in the literature for several invertebrate species exposed to different chemicals using Bayesian inference (R and JAGS softwares). This preliminary attempt suggests that considering the biotransformation process simultaneously to other processes in a generic TK model is feasible, as it was applied to several invertebrate species and chemicals. The Bayesian approach is of real added value to estimate model parameters and to access to a more accurate assessment of uncertainty around estimates and thus TK model predictions. We will further consolidate this attempt with toxicokinetic data from an ongoing experiment in our laboratory involving pyrene, its metabolite 1-OH-pyrene and the amphipod *Gammarus fossarum*.

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Biotransformation Leading to Increased Toxic Metabolites: an Overlooked Risk in Aquatic Organisms?

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Numerous micropollutants have been detected concurrently in aquatic systems. Biotransformation plays a critical role in the bioaccumulation and toxicity of these chemicals by altering their hydrophobicity and toxic potency to certain species. This study aimed to elucidate how biotransformation change the toxicokinetics and toxicity of parent compounds and to gain a better comprehensive understanding of the risk for the aquatic environment. Therefore, we first investigated bioaccumulation and biotransformation of the widely used nonsteroidal anti-inflammatory drug diclofenac in two representative aquatic invertebrates, i.e. *Gammarus pulex* and *Hyalella azteca*. Second, we compared acute toxicities and toxicokinetics of parent compound diclofenac and its major metabolites in *G. pulex* and *H. azteca*. Furthermore, we validated its widespread occurrence in other chemicals and other aquatic organism (fish *in vitro*) at environmental relevant level. Diclofenac methyl ester was the primary metabolite in all three species. The methylation was also observed for triclosan and bezafibrate with distinct structures and was further determined in organisms exposed to environmentally relevant levels of chemicals, suggesting that methylation might be a general process in aquatic organisms. Bioaccumulation factors (BAFs) of diclofenac methyl ester were 229 L kg_{ww}^{-1} and 84 L kg_{ww}^{-1} in *H. azteca* and *G. pulex*, which were 168-458 times higher than the parent compound diclofenac in these two species. This can be explained by the higher hydrophobicity (log D_{ow} = 0.9 for diclofenac; log D_{ow} = 4.4 for diclofenac methyl ester at pH 7.9). The LC_{50}s of diclofenac methyl ester was decreased by 3 order of magnitude (432 times) to 0.53 L mg^{-1} in *H. azteca* in agreement with the higher bioaccumulation. Toxicokinetic modelling in *H. azteca* suggests that the secondary major BTP diclofenac taurine continuously accumulated in the organisms and resulting in prolonged half-life. This could be explained by ion trapping of the ionized diclofenac taurine. Our results show that methylation of diclofenac forms diclofenac methyl ester, which leads to higher bioaccumulation potential and exerts higher toxicity to aquatic invertebrates. In addition, diclofenac taurine is highly accumulated in *H. azteca*. The findings suggest that biotransformation leads to more hydrophobic compounds which should be taken into consideration for future risk assessment.

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Integrating bioaccumulation assessment tools for mammals: from data collection to in silico modelling

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Toxicokinetic (TK) processes such as chemical absorption, distribution, biotransformation and excretion (ADBE) are fundamental to better understand relationships between external and internal exposures and to assess bioaccumulation in mammals. The potential biotransformation rate of a xenobiotic can be estimated using *in vitro* tests to obtain the *in vitro* intrinsic clearance (CL_{int}). Then *in vitro-in vivo* extrapolation (IVIVE) models and QSARs (Quantitative Structure-Activity Relationships) can be used to maximize the information available from *in-vitro* and *in-vivo* measurements. The integration of these different data streams in a coherent framework for the assessment of mammalian bioaccumulation is one primary objective of the CEFIC LRI ECO44 project “Integrating Bioaccumulation Assessment Tools for Mammals (iBAT-Mam)”. This work presents initial efforts to assimilate and analyze *in vitro* TK data to develop new QSARs models to predict biotransformation rates in humans. A new database of >11000 human in vitro biotransformation rate estimates derived from microsomal, S9 homogenate, and hepatocyte-based assays for >8500 organic chemicals was recently developed and critically evaluated. In the present study we conduct in-depth statistical analysis of the data and explore the possiblity of QSARs. Multivariate analysis identified structural patterns in the highly heterogeneous dataset. Principal Components Analysis revealed a good overlap of all assay media that have a similar distribution in the space defined by the theoretical molecular descriptors. Cluster Analysis was performed in order to divide the data into different structural groups. We obtained QSAR models for the different structural groups for hepatocyte and microsome media; all models are stable, robust (R^2>0.75 and Q^2_{loo}>0.70), externally validated and predictive when they are applied to generate predictions on different splitting schemes. Particular attention was given to the structural applicability domain (AD) of all the proposed models. These results demonstrate that QSAR methods can predict biotransformation rates for humans to improve bioaccumulation assessment which is important in the scientific evaluation of risk chemicals may pose to humans and the environment and is a current focus of regulatory effort. These new QSARs can also be used to extrapolate emerging *in vitro* bioassay data from programs like ToxCast, Tox21 and SEURAT to *in vivo* exposures corresponding to *in vitro* bioactivity.

Individual Variation in Ecotoxicological Research: a Change from Unwanted Noise to a Meaningful Endpoint

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Individual variation in ecotoxicology - not only unwanted noise but a factor affecting population tolerance

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Although variability is of major importance when measuring the tolerance of organisms to unfavourable environments, its importance in the survival of organisms to unhospitable environments is seldom studied in ecotoxicological research. In fact, variability is usually addressed in statistical testing only to evaluate, if the variances of different experimental groups are similar or not. If variances do not differ statistically from each other, parametric statistical testing can be used to compare means. If variances differ significantly, different transformations are used to decrease the variance differences to levels, which are acceptable for parametric statistical testing. If even transformations do not make variances statistically non-significantly different, non-parametric statistical testing about the differences between the means must be used. The whole treatment has as an outset that any change of variability is just unwanted noise, and that the only important thing is to compare the means. However, changes in variability can determine, if some organisms can tolerate the unhospitable conditions or not. Further, if the differences in variabilities between groups were only experimental error, the question must be asked: why would there be a different error in the groups, when the same methods by the same scientists are used throughout the study? We have evaluated, if ecotoxicological studies commonly have differences in the variability between treatments. Notably, it is possible that a response in variance can be seen even if the mean does not change significantly. If such a case is interpreted from the mean-centred outset, nothing happens, although the change in variability can affect the survival of some individuals of the population.

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Variability in response between individuals and populations of fish in response to chemical exposures

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Fish populations in natural ecosystems are exposed to a wide range of environmental stressors, from changes in biotic and abiotic factors to complex mixtures of chemical pollutants. Individuals within these populations can be exposed continuously or intermittently over their life cycle and their susceptibility to stress is dependent on multiple factors including their genetic makeup, exposure history, stage of the life cycle and health of the individuals at the time of the exposure. As a result, the response to a stressor will likely be variable between individuals within a population and also between populations. In addition, exposure to stressors can cause changes in the variability of certain parameters in the exposed population, reflecting the differential threshold for response in different individuals. Therefore, variability in the responses to stressors between individuals and populations is likely to be an important component of the mechanisms responsible to population survival in the wild. We will present examples of the variation in response to chemical stressors in fishes and discuss their likely causes. These include increased tolerance to metals in brown trout inhabiting a river with extremely high metal pollution, likely as a result of genetic adaptation; and increased tolerance to copper in a population of stickleback previously exposed to this stressor during embryogenesis, possibly mediated by epigenetic mechanisms. In addition we will discuss the impact of variable husbandry conditions on the response to chemical toxicants. Finally, we will show how exposure to copper increased the variability in Pcrit, a measure of adaptation to hypoxia, and supressed the ability of fish to acclimate to low oxygen availability in the water. These examples document the importance of considering the variation in response to stressors and its causes as part of understanding how populations of fish survive contaminated environments.

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Physiological plasticity in changing oceans: lessons from marine bivalves and corals

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It is well known that species use diverse life strategies (growth, reproduction, survival), but the mechanisms that promote and regulate this diversity remain unknown. Furthermore, elucidating the way that regulatory processes that define these paths operate under environmental pressures to control animal responses and adaptive reactions is a challenging task in environmental studies. Physiological plasticity, which describes the process of tuning organismal phenotypic traits to varying environmental conditions, within their lifetime, is at the base of a species tolerance to environmental challenges. In this regard, variations in mRNA expression profiles are explored as early-warning molecular markers of physiological plasticity and are suggeste to represent an important source of information on regulatory mechanisms governing stress responses and acclimatory processes. In this context, studies on marine invertebrates offer a remarkable opportunity to investigate how physiological plasticity may influence animal responses triggered by environmental pollutants and climate changes. We report on our ongoing research activity in which we use mussels and scleractinian corals as model organisms to infer physiological plasticity and its influence on the responses triggered by environmental changes in the marine environment. Mussels and corals are sessile calcifying organisms with simple body architecture but endowed with complex molecular and physiological mechanisms allowing them to efficiently cope with coastal marine environments where they are exposed to fluctuating environmental parameters and elevated levels of natural and anthropogenic pollutants throughout their life-cycle. We report results on the influence of physiological plasticity on mussels’ and corals’ capability to cope with environmental challenges under *in situ*(baseline physiological status) and laboratory (stress responses) conditions, allowing us to discriminate physiological and/or life history traits promoting stress tolerance and adaptation in a global change scenario. Understanding the underpinning molecular processes can contribute predicting the future of shallow-water calcifying organisms under projected scenarios of anthropogenic-induced global change

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Context-dependent repeatable behaviour in copepods -consequences and approaches for ecotoxicology
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In ecotoxicology, we often ignore responses of individuals and focus on average responses. Yet, both terrestrial and aquatic animals display consistent behavioural differences between individuals, in other words, "personalities". The distribution of behavioural differences within a population contains vital information for predicting population responses to novel environmental challenges. Currently, individual data for behavioural and physiological traits of small marine invertebrates are few, partly because such variation is lost within published group means and assumed normality, but also because acquiring individual data at reasonable sampling sizes can be a challenge. In toxicology, phenotypic variation is further reduced by using single strains and clones to the effect of (single) toxicants. We used an automatized imaging system to test the combined effects of copper and kairomones on swimming activity levels and development in the marine copepod *Tigriopus brevicornis*. While direct stress effects on swimming activity were weak, individuals behaved consistently different. The individually different behavior was only expressed when under the influence of kairomones, and not under copper exposure alone. This pattern indicates that copepods express repeatable, but context-dependent, behaviour. It further shows that variation in behavioural and probably other data can hide consistent differences between individuals, which could have implications for the adaptability and the ability of populations to cope with environmental challenges. In toxicology, this affects the understanding on toxicants' effect, and the realism in extracting knowledge from a laboratory monoculture to a more realistic situation including biological variation. We further tested a similar automated imaging system that can accommodate both aquatic and terrestrial small invertebrates to measure differences in developmental growth pattern. The temporally-highly resolved growth data revealed previously unidentified importance of pedigree and differences between individuals for coping with environmental stressors.

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Variation of polychlorinated biphenyl congener profiles in the UK harbour porpoise (Phocoena Phocoena) in relation to age, gender and geographic location
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Polychlorinated biphenyls (PCBs) are persistent and lipophilic chemical compounds that pose a health risk to humans and wildlife. Harbor porpoises are long-lived and feed at a high trophic level and therefore accumulate some of the highest levels of PCBs in wildlife. Studies typically use the sum of PCB congener concentrations to investigate PCBs in wildlife. However, less work has been done to investigate congener profiles and the factors that influence variation between individuals. It is important to study individual congeners because their toxicological effects and thresholds can vary. Interactions between congeners can cause the magnitude of their effects to increase. Hence, toxic effects of mixtures can occur at levels below the summed effect levels for the component congeners. We found congener profiles vary with age, sex and location. Significant variation was observed between adult females and subadults which is likely to be a consequence of pollutant offloading between mothers and calves. This is important because animals are receiving this dose at a time when they are most vulnerable to its effects. Understanding more about variation in congener profiles is an important step towards better understanding the potential effects and threats of PCB exposure in cetaceans.

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Genetically diversified but morphologically identical: are cryptic lineages comparably sensitive?
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Ecotoxicology assumes that traits of individual species (such as sensitivity) only vary around one mean value. However, this assumption may not be applicable in the case of genetically distinct but morphologically identical lineages within nominal species (i.e., cryptic lineage complexes). This is because of a genetic differentiation among lineages comparable to an interspecific level and thus distinctly evolved biological traits that may influence lineages' response to environmental change. Thus, any generalization about cryptic lineages' traits on the basis of only one assessed lineage may be questioned. Hence, we tested whether two cryptic lineages – namely lineages A and B – of the freshwater amphipod *Gammarus fossarum* (Koch) differ in their sensitivity towards chemical stress using the fungicide tebuconazole (up to 600 μg/L), the insecticide thiacloprid (up to 50 μg/L), and ammonia (up to 1.3 mg/L) as model stressors. Gammarids were exposed individually towards the stressors (n=15-20) for 5 to 7 days, while their feeding rate on leaf discs was used as a measure of sensitivity. Results showed a biologically meaningful and statistically significant higher sensitivity towards chemical stress for individuals of cryptic lineage A compared to their counterparts of cryptic lineage B. The extent of sensitivity-differences between lineages was dependent on the type of stressor, with an up to six-fold and two-fold higher sensitivity of cryptic lineage A when applying pesticides and ammonia, respectively. Potential confounding factors that could have influenced the outcome of the studies, such as the season of collection, gammarids' life stage, parasitism, and physiological fitness as well as the upstream land-use patterns at the sampling sites, either were actively controlled for or showed only minor deviations between the assessed lineages. Consequently, the trait differences observed in our studies were most likely explained by the considerable genetic differentiation between the assessed cryptic lineages. Although we only concentrated on one single trait (i.e., sensitivity), our studies indicate meaningful implications in the reliability and quality of environmental monitoring and ecological risk assessment, if cryptic lineage complexes – also reported for standard test species such as *Lumbriculus variegatus*, *Eisenia fetida*, and *Hyalella azteca* – are ignored.

Improving Decision Support for Sustainability Going beyond Standard LCA (III)

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The Food Loss and Waste (FLW) Value Calculator: addressing challenges in the interpretation of nutrition and environmental impacts of FLW to improve decision support
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The FAO estimates over 30% of produced food is lost or wasted -- never reaching the mouths of consumers. Food Loss and Waste (FLW) thus poses a massive food system sustainability issue as well as a nutrition and food security issue. Questions remain however regarding how in practice FLW should be addressed in order to improve food system sustainability and food security. There are a variety of decisions that can be made by various stakeholders, for example: reducing or tailoring food production, applying business solutions to reduce food loss and waste at various stages across the value chain, donating to food banks, upcycling or reusing wasted food for different industrial purposes. In order to aid decision-support Quantis sustainability consultants created the Food Loss and Waste Value Calculator. The Calculator was released in its beta version in October 2018, by means of the support of the Food Reform for Sustainability and Health (FReSH) program of the World Business Council for Sustainable Development (WBCSD), and close collaboration with the World Resources Institute (WRI). The purpose of the Calculator is to quickly screen the impacts of food loss and waste with respect to its environmental impacts and nutrition in order to support science-based decision making. Through interactions with various entities regarding the calculator, major challenges regarding the interpretation of FLW impacts have emerged. For example, if feeding FLW to animals creates an environmental impact offset, how should this be interpreted? In this presentation we will discuss the practice of addressing the challenges of interpreting the impact of FLW and the implications for decision support in various contexts.

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Environmental impact variability of French organic wheat
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Studying agricultural systems in LCA often comes with the question of studying variability. Agricultural production systems are usually more diversified than other production systems because of a wide variety of (1) farming practices and (2) emissions which vary depending on the local context. Identifying a representative system at a large geographical scale (e.g., a country) is therefore difficult. AGRIBALYSE is a database of Life Cycle Inventories (LCIs) of the main French agricultural products at the farm gate. Its first version contains mainly representative LCIs of French conventional agricultural systems. Creating these LCIs required compromises in data collection and emissions modelling. We are now creating LCIs of French organic agricultural systems for AGRIBALYSE. Compared to conventional systems, organic systems tend to have more diverse farming practices and depend more on soil and climate conditions as they do not use synthetic inputs. In the project "ACV Bio", we are attempting to capture the diversity of these systems. In this study, we explore the variability in environmental impacts of 18 organic wheat crops that differ in farming practices (e.g., cropping systems, fertilisation, tillage) and local context (e.g. location, soil production potential). Emissions were estimated using models recommended by the AGRIBALYSE database and calculated using the MEANS-InOut tool. We used the characterisation methods ILCD 2011 Midpoint v1.09 and Cumulative Energy Demand (CED) v1.09 to calculate midpoint impacts. PCA and HCPC were then performed as a function of 6 practice-related variables and 7 impact-

related variables. Practice-related variables were place in the cropping system (CS), duration of the CS (years), yield (t DM/ha/yr), total nitrogen (N) input (kg N /ha/yr), tillage operations (number) and ploughing operations (number). The preliminary results suggest that (1) the longer the duration of the CS, the lower the freshwater eutrophication and ecotoxicity impacts, and (2) the higher the yield, the lower the climate change, acidification, marine eutrophication, land use and CED impacts. We are currently assessing the variability in impacts of individual crops and CS per ha of land occupied. Moreover, other impact categories such as biodiversity or human toxicity should be added to better assess the variability in impacts of French organic agriculture. Overall results will be used to identify options to reduce impacts of these crops and systems.

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Evaluation of plant protection strategies with LCA and risk assessment for five crops in Switzerland
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The use of pesticides is increasingly debated due to unwanted side-effects on humans and the environment. To inform farmers how to reduce such side-effects, comparative assessments of risks and environmental impacts of different crop protection strategies are required. In the present study, crop protection scenarios with different treatment intensity (LOW, MEAN, HIGH) were analysed for five crops in Switzerland: rape seed, wheat, carrots, potatoes, and sugar beets. The MEAN and HIGH crop protection are based on the 50th and 75th percentile of the treatment frequency per pesticide category using a dataset from the Swiss agricultural environmental monitoring, while the LOW scenario represents a reduced treatment frequency, according to the guidelines of the label organization IP-SUISSE. The analysis was conducted with Life cycle assessment (LCA) and risk assessment (RA) method in parallel. LCA assesses average long-term impacts over the whole life cycle, while RA focuses on maximum effects in the short-term, which are site- and context-specific. For LCA, the transfer of pesticides into different environmental compartments was modelled with the model PestLCI. The aquatic and terrestrial ecotoxicity were assessed with USEtox V2.02 and ReCiPe 2016, respectively. The analysis of the LCA results was carried out in two steps: first, only the impacts of pesticides were analysed for the cultivation of 1 ha of crop, in order to ensure a comparability with RA results. Second, a full LCA was conducted (functional unit 1 kg fresh product), including the entire life cycle of the crop. The RA was conducted with the model SYNOPS. It allows a comparative evaluation of overall risks to the field-adjacent local environments and can be used for assessing individual treatments as well as entire treatment sequences. This study showed that a considerable reduction potential exists for pesticide treatment patterns, particularly for the situation with high treatment frequency. Only one or a few active ingredients dominated ecotoxicity impacts and risks. Avoiding these dominating active ingredients seems to be promising for mitigating ecotoxicological impacts. Furthermore, the study showed that it is necessary to consider all relevant environmental compartments and not to focus on water bodies alone. The parallel analysis of environmental impacts (LCA) and risks to the environment (RA) provided a more complete assessment and allows for a robust decision support.

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Geolocated Life Cycle Assessment modeling to evaluate the environmental performance of emerging renewable energy technologies: Application to floating offshore wind farms
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The large-scale development of renewable energy systems (RES) to satisfy the increasing energy demand and replace non-renewable fossil sources while reducing the environmental impact represents a major challenge from both socio-economic and environmental perspectives. Although RES are expected to have low resource consumption and pollutant emissions during the electricity production phase, manufacturing and end-of-life phases should be accounted for to ensure their environmental benefits compared to other energy systems. In this study, we propose the use of a geolocated Life Cycle Assessment (LCA) to evaluate the environmental performance of emerging RES. The approach is applied to a pilot project of a floating offshore wind farm to be installed in the Mediterranean Sea. Since the environmental performance of RES is generally expressed as the ratio of the total impact of the technology over its life cycle to the corresponding electricity production, accurate estimates of both terms are essential for a reliable LCA. The total environmental impact over the RES life cycle strongly depends on the quality of data collected during the inventory phase. In this work, the material flows were mainly provided by direct measures from the project suppliers, which ensure the reliability of input data for the model. However, even if the total impacts are based on high-quality inventory data, the environmental performance can be to a large extent affected by uncertainties related to the electricity production. In the case of emerging RES, estimating this production may be particularly challenging due to the absence of historical data for existing installations, especially when dealing with variable resources such as wind or solar irradiation. For such systems, we have developed an approach that consists in parameterizing the electricity production component of the LCA model and coupling this parameterized model to existing geolocated meteorological databases to estimate the RES environmental performance in different locations before its installation. The proposed approach was applied to the floating offshore wind farm 1)to compute the environmental performance of the system accounting for the geolocated electricity production and 2)to identify the main parameters contributing to the evaluated impact categories. Additional sensitivity analyses showed the critical role of the lifetime and the wind data source as the main parameters influencing the environmental results.

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Emissions of electric vehicle charging in Europe in future scenarios: The effects of time of charging
A.A. Arvesen, S. Völler, C. Hung, Norwegian University of Science and Technology; V. Krey, International Institute for Applied Systems Analysis; A. Strømman, NTNU The Norwegian University of Science and Technology / Energy and Process Engineering Industrial Ecology Programme; M. Korpås, Norwegian University of Science and Technology
Switching from petroleum-burning vehicles to electric vehicles powered by climate-friendly electricity is an important strategy to stabilize the climate. Understanding the current and future emissions associated electric vehicle charging is a key component to formulating effective electric vehicle strategies. At the same time, developing such an understanding is a challenge: Among other things, this is because of the the location- and time-specific character of both electricity generation and vehicle charging, and to uncertainty surrounding future electricity generation mixes. The importance of time can be expected to increase in the future as electricity generation from variable and weather-dependent energy sources (i.e., wind and solar) increases. Here, we use a computer modelling approach in order to elucidate the effects of charging time (time of day) on the greenhouse gas emissions of electric vehicle charging. To do so, we combine climate stabilization scenarios that describe long-term evolutions and aggregated characteristics of power systems, detailed power system modelling that determines energy balances (supply, consumption, trade, losses) at an hourly time resolution, and future-oriented life cycle analyses that quantify direct and indirect emissions of electricity. We investigate three hypothetical charging profiles for electric vehicles, namely fixed charging, workplace charging and home charging. We explore a range of electricity generation scenarios that rely with different degrees on wind, solar, nuclear and natural gas energy. We investigate questions such as: What are the emissions of electric vehicle charging in future scenarios for Europe? How do the emissions vary depending on time of charging (time of day) and country? Does the consideration of time of charging increase or decrease emissions estimates, compared with quantifications that do not consider time of charging? What combinations of electricity mixes and vehicle charging profiles yield the lowest emissions footprints of charging? How do results differ depending on whether average or marginal emissions from electricity are used? Finally, what are the emissions reduction opportunities of 'smart' charging regimes, such as charging at daytime when solar electricity generation is at a maximum? Results, discussion and conclusions will be available for the conference.

Gender Bias and Equal Opportunity in Scientific Research

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Promoting equality in research organizations: lessons from the Baltic Gender project
H. Valve, Finnish Environment Institute / Environmental Policy Centre
In the field of environmental policy and science, heterogeneity and the inclusion of diverse competences is essential. This is one reason why there is urgent need for the removal of discriminative barriers in academia. In part, the discriminative barriers have to do with gender and with the capacities of women to have an impact on research. The on-going Baltic Gender project (Horizon 2020) approaches these barriers from several perspectives. It recognizes that the lack of family-friendly arrangements, unbalanced research networks, unwritten norms and established routines can, among others, produce and maintain inequality. As a male dominated field, marine science and innovation provides a fruitful context for the development and testing of good practices. In my presentation I will share findings and experiences from the project. The key aim is to initiate discussion about the relevance of the lessons for the SETAC community and get feedback to the proposed measures and approaches. The focus will be on two topics and

working areas in particular. First, I will present findings about the promotion of structural change in the research institutions participating in the Baltic gender project. Best practice measures identified by Kiel University point to the innovative means by which women's academic careers and participation in academic decision-making can be promoted. In the second part I will address the integration of gender in the content of marine research. The very practicing of science can matter for gender equality. In the Baltic Gender project, a method protocol is being produced to support researchers to analyze how their projects link to gender and to promotion of gender equality.

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Gender bias, publishing, and SETAC

J. Lynch, T.H. Schlekat, SETAC

The focus of this talk will be on three forms of gender bias experienced by women while publishing their research: reviewer bias, authorship bias, and citation bias. We will share personal accounts of perceived bias in publishing from SETAC members and strive to determine whether these are merely anecdotal or indicative of a more pervasive issue. We will review the literature on gender bias in scientific publishing and examine the data from the SETAC publication outlets *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM) to evaluate if they support the narratives. Using the information gathered, we will report on and propose solutions, where appropriate, to help overcome bias against women with regards to publishing in the scientific fields in general, and more specifically in SETAC journals. The review of the literature indicates that large scale reviewer surveys, such as the recently published Publons 2018 Global State of Peer Review, have found that women are underrepresented in peer review. Women account for less than a quarter (22%) of peer-reviewers registered with Publons, a peer-review recognition website with over 515,000 researchers, and a recent analysis by the American Geophysical Union showed that male authors recommended women reviewers 18% of the time, while male editors recommended women reviewers only 16% of the time. Further, the literature shows disequilibrium in gender ratios among authors who contributed equally and suggests that inequities in credit sharing may be a contributing factor to the continuing gender imbalances reported for academic positions, grant funding, and awards. Preliminary interviews with SETAC members reflect suspected gender-bias in publishing, though not necessarily in SETAC journals. A review of the editorial composition at the journals shows that the current ratio of male to female senior-level editors at ET&C and IEAM are 4:1 and 3:1, respectively. However, preliminary publication data from 2014–2018 indicates that female first authors at ET&C are on par with, and may exceed, male first authors. The talk will report final results based on publication data through the end of 2018.

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Old and New Faces of Gender and Diversity Bias in Science

M.L. Diamond, University of Toronto / Department of Earth Sciences; A.O. De Silva, Environment and Climate Change Canada

"Its as if I didn't exist – he looked right past me even though I was chairing the meeting". "His comments and actions made me feel uncomfortable but I found it difficult to respond in a way that protected my dignity". In this paper and discussion, we explore some of the multi-facetted experiences of women in environmental science and in academia. The statistics of women's participation in the academy, at least in Canada, paint a positive picture. Women now dominate the graduates of numerous science and a few applied science programs. Women comprise 40% of full-time academic teaching staff in Canadian universities which is an increase since 2010. The salary gap is narrowing, with median female faculty earning 12 vs 25% less than that of their male counterparts in 1990 vs 2016, with the gap depending on rank. Most women experience no or minimal gender bias as they move through undergraduate and graduate school. However, statistics on women's participation continue to fit the "scissor" effect of attrition through the ranks. Of concern is that visible minorities and people of colour constitute only 21 and 2% of all faculty in Canadian universities, respectively. Women of colour have reported disproportionately higher difficulty in achieving insider status, receiving effective mentorship, and building a collaboration network. They also report higher rates of experiencing cultural alienation and a greater accumulation of disadvantage and under-estimation of performance. For about 20 years women chemists at SETAC North America have been meeting to share our accomplishments and challenges. We have built a women's network that helps junior to senior women advance our careers through mentorship and other supports. We share our experience with this network and other actions that we have taken to make environmental science welcoming for all.

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Round Table Dialogue

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Environmental Monitoring and Risk Assessment of UV Filters in Aquatic Environments

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Concentration of organic UV filters in surface seawater, coral tissue (Porites spp.) and sediment samples from Oahu, Hawaii

C.L. Mitchelmore, University of Maryland / Chesapeake Biological Laboratory; K. He, University of Maryland, Baltimore county / Chemical, Biochemical & Environmental Engineering; E. Hain, University of Maryland Baltimore County / Department of Chemical Biochemical and Environmental Engineering; A. Feerick, University of Maryland Baltimore County; R. Younger, UMCES-CBL; A. Heyes, University of Maryland; M. Gonsior, University of Maryland / Chesapeake Biological Laboratory; A. Conway, UMCES-CBL; L. Blaney, University of Maryland Baltimore County / Department of Chemical Biochemical and Environmental Engineering

Concerns over the toxicity to corals of some organic UV filters contained in sunscreen products resulted in the banning of oxybenzone (BP-3) and 2-Ethylhexyl 4-methoxycinnamate (EHMC or octinoxate) in Hawaii, USA. However, limited or no information is available on the environmental concentrations of these chemicals in seawater, sediment and coral tissue from Hawaiian coral reefs. To address these knowledge gaps surface seawater, coral tissue (*Porites* spp.) and sediment samples from multiple sites (n=29) in Oahu, Hawaii were analyzed for 13 organic UV filters. Surface seawater and sediment samples were collected from a variety of State Parks around Oahu, including Hanauma Bay. A more detailed study of the concentrations of UV filters in seawater, sediment and coral tissue was focused at Waikiki Beach (popular tourist destination), Kaneohe Bay and Ka'a'awa (chosen as a 'reference' location). UV-filters were measured using LC-ESI-MS/MS techniques. Up to nine UV filters were detected in seawater and sediment and up to eight detected in coral tissue samples, although there was no correlation between the concentration of UV filters detected in each environment matrix. The highest detection frequencies and concentrations of UV filters in all matrices was homosalate (HMS), octisalate (OS), BP-3 and octocrylene (OC) although their concentration order depended on the matrix. BP-3 was ubiquitous in surface seawater as it was detected at all sites with the highest concentrations found in the late afternoon samples from Hanauma Bay, Waikiki Beach and Ko'Olina Beach Park, however, at 15 of the 29 sites, aqueous concentrations were less than 10 ng L^{-1}. Similarly, BP-3 was detected in all coral tissue samples at concentrations of 17-265 ng g^{-1} (dry weight, dw.). However, BP-3 was only measurable in sediment samples at 16 out of 29 sites at < 4 ng g^{-1} (dw.). No quantifiable levels of EHMC were recorded for the seawater or coral samples, although OCX was quantified in sediment samples from Kaneohe Bay at concentrations less than 13 ng g^{-1} (dw.). This study provides insight as to the environmentally relevant concentrations in seawater and sediment in Hawaiian coral reef locations. In addition, this study is the first to report concentrations of UV-filters in corals from the USA. These data provide an important baseline for future risk assessments of the potential toxicological effects of UV-filters on coral reefs and other marine organisms in Oahu, Hawaii.

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UV filters: can we estimate the risk to coral reef?

V. Poulsen, LOREAL / Environmental Safety; M. Leonard, J. Fel, LOREAL / Environmental Research; S. Mezzache, LOREAL / Chemical Analysis

UV filters used in cosmetics are currently under scrutiny due to published papers relating their toxicity towards coral and their potential impact on coral reef bleaching. It is recognised by experts that the cause of coral bleaching is multifactorial, mainly due to global warming, overfishing, and marine pollution. Conscient of the its role and responsabilities, L'Oréal developed, in collaboration with the Monaco Scientific Center, a test on coral nubbins of the species *Styllophora pistillata* to assess the impact of UV filters, and derived NOEC values after a 5-week exposure. When comparing these values to published monitoring data in marine environment in coral reef areas, the obtained ratios show a margin of safety between 692 and 1963 for oxybenzone, and more than 119000 for octocrylene. Avobenzone was not detected in marine water. When comparing these ratios to assessment factors that are usually used in regulatory risk assessment (e.g. 1000 for acute toxicity and 100 for chronic toxicity in REACh regulation), this allows to conclude on negligible risk for coral reefs for octocrylene and avobenzone, and moderate to low risk for oxybenzone. For the other UV filters, there is either a lack of effect data or a lack of measured environmental concentrations to derive these ratios and conclude of potential risk to coral reef.

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Ecotoxicology of mineral-based sunscreens on reef-building corals

A. Tagliati, Heriot Watt University / Institute of Life and Earth Sciences; S.J. Hennige, The University of Edinburgh / School of Geosciences; T.F. Fernandes, Heriot-Watt University / Centre for Marine Biodiversity and Biotechnology, Institute of Life and Earth Sciences; J. Labille, CNRS; L. Hedouin, CNRS CRIOBE

While several studies have recently demonstrated the toxicity of *chemical* sunscreen compounds on tropical corals, the impact of *mineral* sunscreens and the other core ingredients in sunscreen that constitute the oil phase (emulsifiers and emollients), have not yet been studied. Here we present an integrated view of the effects of custom-made sunscreen lotions, differing in the type of emulsifier ingredients and titanium dioxide nanoparticles ($nTiO_2$) used as UV filters, on the sea anemone *Exaiptasia pallida* (a coral model organism), and early-life stages and mature corals, to identify the sunscreen compounds having the least harmful effects. Sea anemones photosynthetic activity and heat shock proteins (HSP70, HSP90) gene expression were investigated, along with coral respiration, photosynthetic efficiency, symbiont density, chlorophyll a concentrations, gamete fertilization success and larvae survival at multiple timepoints. Coral respiration and net photosynthesis are impacted by sunscreen released in seawater, but sunscreen toxicity does not depend on the action of UV filters alone. Sea anemones showed a negative impact on their photosynthetic activity even when exposed to filter free formulations, indicating that the sunscreen oil phase is also responsible of the observed toxicity. Indeed, gametes exposed to sunscreen formulated with an organic emulsifier (cellulose nanocrystal) showed a reduction in abnormally developed embryos. Results from the present study highlight the importance of taking into account emollient and emulsifier ingredients, in addition to UV filters, when assessing the toxicity of sunscreen products and are promising in identifying the least toxic cosmetic ingredients in order to develop environmentally friendly sunscreens.

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NanoScreen - Minimizing the risk associated with nanomaterials used in sunscreen at all lifecycle stages

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Among cosmetics and personal care products, sunscreen products are of emerging concern regarding both environmental and human healths. While some organic UV blockers have been evidenced to undergo rapid photodegradation, to induce allergene skin reactions due to dermal penetration, or to cause deleterious effects on marine system, the fate of mineral UV blockers is still under consideration from a regulatory perspective. This is largely related to the potential impact of nanotechnology-based products on both environment and human health. The nano-TiO_2UV-blockers typically used in sunscreen usually consist of rutile nanoparticles coated with a first mineral layer of silica or alumina aimed at blocking the photocatalytic character, and thus passivating the nanomaterial. In addition, the grafting of a second layer of organic coating is aimed at favoring the nanomaterial dispersion in the cream formulation. Once drained from the skin either through bathing activity or everyday usage and cleaning, the nanomaterials contained in the susncreen can be released to the sea shore. Their behavior in this system is largely determined by this industrial coating and by their initial dispersion in the formulation. This project aims to develop the Eco-design of sunscreens through the minimization of risks associated with nanomaterials incorporated into the formulation. The release and the impact on the marine environment are mainly considered and studied using the following approaches. In order to estimate the release of nanomaterials from sunscreen in marine environment and the subsequent bioaccessibility and exposition to the living organism, we carried a field campaign on three beaches on the french coast. The titanium concentration was measured in the sea water as a fuction of the number of swimmers. The impact on marine living organism is taken in count by toxicological tests on the sea urchin (Paracentrotus lividus) used as a biological model. In particular, the impact on the sea urchin's immune system (cell viability and toxicity) and reproductive system (embryos development) was evaluated, exposing them to different TiO_2based sunscreen UV filters concentrations. The impact of TiO_2NPs/Oil emulsions on this biological model was also considered.

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Evaluating the ecotoxic versus beneficial effects of Benzophenone-type UV filters using in vivo and in vitro bioassays

E. Thia; P. Chen, National Taiwan University / Agricultural Chemistry

Benzophenone derivatives (BPs) have been widely used as active ingredients in chemical sunscreens, serving as UV filters which help protect the skin from excessive exposure to sunlight. However, recent studies show that BP-type UV filters (e.g. BP-3) give rise to allergic reactions to mammals and toxic effects to non-targeted organisms. Hence, it is crucial to analyse the toxicity of benzophenone-class UV filters. In this study, several commonly used BPs were first screened for their acute and sublethal toxicities. Medaka (*Oryzias latipes*) larvae were observed over a 96-hour period to determine the acute toxicity of different BPs. Based on LC_{50} data of these compounds, BP-2 was the least toxic among all, followed by BP-3, BP-6, BP-1 and BP-8. Next, the sublethal toxicity was assessed by observing the swimming behaviour of fish larvae. After 24 h exposure, medaka larvae showed no significant difference in the locomotor activity for BP-2, BP-3 and BP-1. In contrast, BP-8 significantly altered the larval locomotion after exposure. This study further evaluated the potential of BPs in inhibiting tyrosinase activity, in addition to their abilities in filtering UV. The whitening potential of BPs was assessed with *in vitro* mushroom tyrosinase assay, using kojic acid as a positive control. Among the BPs evaluated at various tested concentrations, BP-1, BP-3, BP-6 and BP-8 did not show inhibitory effect to tyrosinase enzyme. On the contrary, BP-2 showed an inhibitory effect to mushroom tyrosinase in a dose-dependent manner. Overall, this study indicated that BP-2 was the least toxic compound among all the tested BPs. In addition, BP-2 also showed a promising tyrosinase inhibition activity and may potentially be utilized as a whitening agent. We will further screen the toxicities of more BPs that are commonly used in cosmeceuticals under environmentally-relevant concentrations and continue to investigate the tyrosinase inhibition potential of these BPs using *in vivo* zebrafish (*Danio rerio*) bioassays.

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Using Eco-epidemiology to Assess the Potential Risks of UV Filters to Corals

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A recent study in Archives of Environmental Contamination and Toxicology (Downs et al 2016) indicating potential ecotoxicity issues for coral exposed to UV filters, such as benzophenone-3, has gained a global-level of visibility. This single study has provided laboratory evidence that calls into question the sufficiency of environmental risk assessments associated with benzophenone-3 via sunscreen use, particularly for swimmers and sunbathers. For sub-tropical and tropical climates, the potential occurrence for exposure of BP-3 may be year-around. Spatial coincidence of BP-3 exposure and marine ecosystems highly dependent on corals amplifies the potential issues highlighted in the Downs et al study. However, coral reefs have been shown to be adversely affected by numerous other chemical, biological and physical stressors, ranging from local to global scales. Hence, the protection of corals requires a multi-faceted approach that considers not only potential chemicals stressors, but physical stress – including temperature and changes in habitat quality. We advocate the use of eco-epidemiology to evaluate the relationships between environmental stressors and ecological status within a realistic ecological context. This approach supports the recognition that ecosystem status is driven by a multitude of physical, chemical and other environmental factors. Since the foundation of the evaluation relies on measured ecological status, recommendations from such an assessment have great potential for decision-making (including regulations) that will yield fruitful management actions. Our initial analysis utilizes data obtained from experts at the University of Hawaii (e.g., Coral Reef Assessment and Monitoring Program (CRAMP) http://cramp.wcc.hawaii.edu/default.htm). Measured UV filter and surrogate exposure data were collected for the island of Oahu from Mitchelmore et al (2018). To date, published works by the CRAMP experts indicate that both natural and anthropogenic factors may influence coral cover and species richness. Importantly, no single factor has been found to serve as a proxy for coral cover. Hence, it is clear that coral cover and species richness is dependent upon many factors. Based on CRAMP data alone, there appears to be a lack of data supporting the hypothesis that UV filters provide an adverse influence on corals. Our study places into context UV filters amongst several physical and chemical factors that potentially affect coral community health.

Applications of Bayesian Network Models for Environmental Risk Assessment and Management

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Quantification of AOP by Bayesian network modelling: linking chemical stress to adverse outcomes in Lemna minor

J. Moe, Norwegian Institute for Water Research (NIVA) / Section for Ecotoxicology and Risk Assessment; W.G. Landis, Western Washington University / Institute of Environmental Toxicology; L. Xie, NIVA - Norwegian Institute for Water Research / Environmental Toxicology; K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment; N. Kotamäki, Finnish Environment Institute SYKE; Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment

Although AOPs has gained international recognition as a systematic approach for capturing existing toxicological knowledge to transparently link mechanistic data to toxicity endpoints, most AOPs are not appropriate for quantitative risk assessment. Quantitative AOPs (qAOP) should define the relationships underlying transition from one KE to the next sufficiently well to allow quantitative prediction of the probability or severity of the AO occurring for a given activation of the MIE. We have started developing a Bayesian Network (BN) model to quantify a recently proposed AOP, which links the mode of action of the model respiratory and photosynthesis uncoupler 3,5-dichlorophenol (DCP) to adverse outcomes in the aquatic plant *Lemna minor*. The BN model is based on data from a laboratory experiment exposing *L. minor* to DCP in 8 concentrations with 3 repeated measurements. The measured response variables include OXPHOS (oxidative phosphorylation), ROS (reactive oxygen species), ETR (electron transfer rate), Fv/Fm (maximum quantum yield of photosystem II), LPO (lipid peroxidation) and number of fronds (leaves). The proposed AOP is a network consisting of three chains with the same chemical stressor (DCP) and AO (fronds number). All AOP components were quantified by discrete probability distribution. The causal links (Key Event Relationships) were quantified as conditional probability tables (CPTs), which determine the probability distribution of a child node conditionally on the probability distribution of the parent node(s). The CPTs were quantified by statistical modelling (dose-response curves) of the experimental data and subsequent simulation of response vaules using the estimated parameters and their uncertainty. The quantified AOP-BN model can be used to predict the probability of an adverse outcome (e.g., the probability of fronds no. below a given threshold) for a given level of the stressor, or for a given state of an MIE or a KE. Moreover, the model can be run diagnostically (backwards) to assess, for example, the most likely range of stressor levels that will cause a specified level of an adverse outcome. Future development of this AOP-BN model can include linking the AO to a population-level endpoint with regulatory relevance, and linking the chemical stressor to an Aggregate Exposure Pathway.

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Integrating Climate Change Stressors and Human Health and Wellbeing into Bayesian Network Relative Risk Model of the Skagit River Watershed
E. Lawerence, Western Washington University / Institute of Environmental Toxicology; L.P. Lackey, Whatcom Community College / Chemistry/Engineering; A.J. Markiewicz, W.G. Landis, Western Washington University / Institute of Environmental Toxicology
Climate change is expected to have widespread impacts on ecosystem services in the Salish Sea. In this research, we focused on the question of how stressors generated by climate change affect contaminant toxicity to species in the Skagit River watershed. Specifically we assessed how those combined effects potentially influence risks to the river's ecosystem services that, in turn, impact human health and well-being. To answer this question, we conducted an ecological risk assessment using the Bayesian network relative risk model (BN-RRM). It is a quantitative, probability-based model that calculates complex relationships between ecological variables to provide estimates of risk to valued receptors (endpoints). We incorporated two main stressor pathways: first, water temperature and dissolved oxygen to represent physical parameters that are influenced by climate change and, second, organophosphate pesticides to represent chemical toxicity and to allow for potential interaction effects between climate and chemical stressors. We focused on Chinook salmon (*Oncorhynchus tshawytscha)* population as an ecosystem service to connect pathways from environmental stressors to human health and well-being endpoints such as human well-being, water quality, salmon fisheries tribal cultural and community health indicators, recreation areas, tourism, boating, and fishing. The BN-RRM enables us to calculate the risks posed by various stressors on these select endpoints in the Skagit River watershed due to climate change. The BN-RRM can also serve as a useful tool for resource managers and decision-makers as part of an adaptive management process and to direct future research efforts in the watershed, as well as in other watersheds in the Salish Sea region.

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The use of Bayesian networks in adaptive management and constructing resilient systems
W.G. Landis, A.J. Markiewicz, Western Washington University / Institute of Environmental Toxicology
We have described the application of the Bayesian network relative risk model (BN-RRM) in adaptive management. The case study used was the South River RCRA site in the western part of Virginia, USA. Harris et al demonstrated that risk to human well being types of endpoints can be conducted using the same basic techniques. In a new publication, Graham et al applied the BN-RRM approach to estimating risk to water quality and community structure endpoints for a series of estuaries in New South Wales, Australia. The Australian case study took advantage of the extensive dataset derived from years of monitoring and the relatively new method of eDNA to establish the conditional probablites of a set of causal pathways. In both case studies the long-term goal is the ability to manage these sites over multiple decades. In applying risk assessment to adaptive mangment we propose three segments. Segment A describes the social, cultural and regulatory factors that are being used as to bound the decision making as towards goals and resources. Part B is the active part of the process, where the research, risk assessment, engineering monitoring, learning and decision process takes place. Part C is the section that observes and reports on the change in externalities to the adaptive system. Climate change can be an example, so can an increase in population pressure, changes in technology, and any other factor that will alter the implementation of Part B. As demonstrated the BN-RRM structure is easily updated with new information, can incorporate management options, and likelihood of different causes can be calculated. The efforts of monitoring plans can be incorporated and the causes reevaluated as an innate part of the adaptive management process. Resiliance can be explored by by adding additional nodes to describe specific externalities or new management tools. The risk can then be recalcuated and the change compared to the original condition. We have found that such efforts find that not all endpoints are similarly affected. For example a managment scheme to reduce Hg contamination to fish alters breeding habitat for Kingfisher birds. We have demonstrated that is it possible to incorporate a Bayesian network based risk assessment into adaptive management. In this playing of what if games the innate advantages of the Bayesian network format becomes a key to developing management plans and examining how they affect resiliency

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Ecological risk assessment of multiple flow and non-flow stressors to the Upper Niger River and Inner Niger Delta, West Africa using PROBFLO.
G.C. O'Brien, University of Mpumalanga / School of Life Sciences; C. Dickens, International Water Management Institute
The millions of people who live in the Upper Niger River and Inner Niger Delta depend on its natural resources and ecosystem services for their livelihoods and wellbeing. The availability of water resources of the region is highly variable, driven primarily by the seasonal flows and the associated flooding of the delta. The aim of this study was to use regional socio-ecological information related to the structure, function, use and protection of water resources in the context of regional management targets, to carry out a holistic regional scale ecological risk assessment addressing flow and non-flow stressors using the PROBFLO method. In this study a review of available water resources information from the study area and region was undertaken. With stakeholders of the study area and real hydrological and hydraulic information, the ten procedural steps of PROBFLO were undertaken. The outcomes for all of the development scenarios considered result in low risk probabilities for most social and ecological endpoints considered, except for the maintenance of the mammal endpoints within the detla itself. In addition, the risk to the endpoints in the delta were relatively greater that the risk to the endpoints upstream in the river habitats. The outcomes of this precautionary desktop E-flow assessment include relatively high percentages of the natural water flows ranging from 39% – 63% across the different regions of the upstream basin to sustain the socio-ecological system. Results show that the maximum planned developments in the catchment for a planned development (FUTURE 1) scenario will result in insufficient water in the river during the dry seasons, that would be unacceptable in the context of the vision of the study area. This will have impacts on the design of new dams and irrigation schemes, as water will need to be allocated to maintenance of the E-flows in the dry season. The PROBFLO method was successfully applied in the case study. From these outcomes, stakeholders have, been able to consider sustainable social and ecological trade-offs between, to balance the use and protection of water resources in the study area. And we have provided the initial requirements for E-flows to maintain the socio-ecological sustainability of the water resources in the region. The adaptability of the approach allows for the incorporation of new information rapidly that will inform adaptive management for the management of water resources in the study area.

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Dioxins in Baltic herring and salmon: an inter-sectoral decision analysis for optimal management of the problem
A. Lehikoinen, University of Helsinki / Ecosystems and Environment Research Programme; P. Haapasaari, University of Helsinki
Ecosystem-based management (EBM) of natural resources requires recognition of the systemic intertwining of ecosystems and human society and an inter-sectoral approach. We used a Bayesian influence diagram to integrate different types of knowledge for evaluating alternative sectoral and inter-sectoral strategies to manage the dioxin problem of Baltic salmon and herring fisheries. The following strategies were evaluated: 1) decreasing dioxin and nutrient loading to the ecosystem, 2) herring and salmon fishing strategies, 3) dietary recommendations, and 4) improved information concerning the benefits of fish eating. In total nine decisions and their combinations were evaluated in the light of three alternative assessment criteria: 1) the dioxin concentrations of Baltic herring and salmon, 2) the human consumption of Baltic salmon and herring, and the associated health risks and benefits, and 3) the commercial value of herring and salmon catches. The results demonstrate the requirement to understand the effects of management measures in a holistic way: managing only one species or policy domain may not be effective, and may also have unanticipated systemic effects in the ecosystem. In general, optimal management depends to some extent on the assessment criteria used, as well as the order in which the decisions are made. Unsynchronized management decisions in different sectors may decrease each other's effectiveness. This implies that to control the dioxin problem as effectively as possible, collaboration between the public health, environmental and fisheries sectors is needed.

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Need to know and need to act: Value-of-information and value-of-contol in Bayesian decision analysis
S. Kuikka, University of Hesinki / ECOENV research program
Bayesian decision analysis is focusing on the decision making under uncertainty. In here, the Bayesian influence diagrams are commonly used tools. Instead of using deterministic equations and point estimate models, they model the dependencies of variable by using conditional probabilities. This allows a relatively simple integration of e.g. economic, social, biological and juridical information in complex decision tasks. An interesting and important part of a Bayesian model is the possibility to calculate backwards from effects to causes, and thereby carry out diagnostic analysis, where the information contents of

several indicators can be integrated to several, potentially competing, uncertain hypothesis which may be causes for the observable effects, often called indicators in biological diagnostics. The value-of-information (VOI) is a well-known concept in Bayesian decision analysis, as the aim of the decision models is to supports selection of preferable actions. It is an estimate of how much one should, as maximum, allocate to improve the knowledge about the system before the management action is carried out. Management action aims to improve the state of the system, whereas the VOI analysis is focused on the improved knowledge. Both aspects are described in one model. The analysis is based on the prior distributions of the knowledge: if this is what I know at the moment (uncertain knowledge described by probability distributions), is there a possibility that the decision will be changed, if I increase the amount of knowledge? This possibility to learn is described by probability distributions. Surprisingly, the concept of value-of-control is poorly known, and very seldomly applied in current applications of decision analysis. It describes how much more probable it is to reach the objectives (increase the value of objective function) if some of the probabilistic variables are changed to be decision variables, which increases the controllability of the uncertain system, described by the conditional probabilities of the model. It should be in the heart of any management analysis, including the evaluation of how likely it is that a new law leads to desired state of the system, in many cases the behavior of humans. It seems that the VOI and VOC are related: there is no need to know if there is no need to act, or if the controllability of the system is poor. I Keywords: Bayesian, decision analysis, value-of-information, value-of-control

One Health: Interdisciplinary Solutions for Complex Problems

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One Health: Interdisciplinary solutions for complex problems - topic overview and session goals

T. Augspurger, U.S. Fish and Wildlife Service / Ecological Services

The topic of One Health is in line with SETAC's mission. One Health is a collaborative, multi-sectoral, and trans-disciplinary approach, working locally, regionally, nationally, and globally, to achieve optimal health and well-being of all animals, people, plants and their shared environment, recognizing their inextricable interconnections. SETAC's mission is to support the development of principles and practices for protection, enhancement and management of sustainable environmental quality and ecosystem integrity. The goal of this overall session is to bring the concept of One Health to the SETAC community. In this introductory presentation, we will provide some background on the One Health framework in the context of SETAC, provide a variety of examples from which the audience can draw parallels to their own research, enhance communication between the different niches within SETAC, and provide a forum to have any relevant questions answered by leaders in the One Health field. This context will help set up set the stage for the following invited presentations. Each of the invited presentations highlights One Health from the perspective of each of the four sectors represented within SETAC; academia, government, non-governmental organizations, and business/industry.

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Advancing integrated holistic approaches for assessment of health and environmental risks within the SETAC scientific community

C. Menzie,

There is great promise in integrating the assessment of human and ecological issues in a manner consistent with One Health. An additional dimension of an integrated approach comes from the social sciences and includes the manner in which risks are perceived and communicated. A holistic view makes sense because environmental decisions are ideally informed by a combination of human health, environmental health, and socioeconomic factors. Considering these in an integrated way rather than as isolated bins of information enables a deeper and more complete understanding of an issue. In particular, identifying the health, ecosystem services, and psychosocial connections between people and their environment yields more complete conceptual models for outlining the nature of an issue. While segmenting the problem into various health and environmental components is necessary for analysis purposes, these segments should flow from explicit recognition that the parts are interrelated. The overall analytical process should track, make use of, and incorporate the interconnections. Many of SETAC's Interest Groups already reflect elements of integrated approaches; a few examples include Ecological Risk Assessment, Human Health, and Ecosystem Services. National and international initiatives have examined how best to integrate human health and ecological risk assessment. The U.S. EPA's Cumulative Risk program seeks to meld human health, ecological and other factors impinging on communities. And, it is evident that an integrated One-Health approach makes sense for developing countries. SETAC members in these countries have pointed to a myriad of health and environmental problems confronting their societies such that a more holistic approach is desired. SETAC together with the Society of Risk Analysis (SRA) is providing an introductory course for the 2019 SETAC Africa meeting that integrates human health and ecological risk assessment. SETAC leadership can make a difference, especially given our inclusive tripartite approach and the deep commitment of scientists in both the developed and developing world who seek collaboration. SETAC is moving forward with global certification for risk assessor. There is great value for risk assessors to develop competence in the full suite of risk factors and human and environmental receptors associated with the issues we are facing. Among these are management of chemicals, introduction of new technologies and products, changes in habitat, availability of clean water, nutrient enrichment, salinization, a host of climate change related alterations, invasive species, and disease. These too are interrelated and consistent with the SETAC Europe meeting theme: One Environment. One Health. Sustainable Societies.

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Extrapolation of Chemical Susceptibilities Across Humans, Domesticated Animals, Wildlife, and Plants using the SeqAPASS Tool

J. Doering, NRC U.S. EPA / Mid-Continent Ecology Division

Chemicals in the environment can cause health concerns for humans, domesticated animals, wildlife, and plants. However, the health of all organisms of concern cannot be pragmatically evaluated and therefore chemical hazard assessment requires extrapolation of information from model organisms to other species. Therefore, the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool was developed by the U.S. Environmental Protection Agency (USEPA) as a rapid, cost effective method of species extrapolation of chemical susceptibilities. Specifically, the SeqAPASS tool compares the primary sequence (Level 1), functional domain sequence (Level 2), or individual amino acid residues at key positions (Level 3) of the protein target of a chemical in a species of known susceptibility to sequences of other species in the National Center for Biotechnology Information (NCBI) database. Sequence similarity metrics are then calculated and results at these three levels of comparison can be used as lines of evidence to predict shared chemical susceptibility across species and taxa. Several case studies have been developed demonstrating the utility of SeqAPASS in species extrapolation of susceptibility to pesticides, human and veterinary pharmaceuticals, as well as for extrapolation of data from mammalian-based high-throughput screening assays. This presentation will demonstrate utility of the SeqAPASS tool to the One Health approach. Specifically, this presentation will outline a case study using SeqAPASS to extrapolate susceptibilities to chemicals that interact with the thyroid axis target, thyroid hormone receptor alpha (THRa), from responses measured in a human-based *in vitro* assay used in the Toxicity ForeCaster (ToxCast) program. Level 1 (primary sequence), 2 (functional domain sequence), and 3 (key amino acid position) comparisons in SeqAPASS all suggest that chemicals that interact with THRa of human will interact with THRa of other vertebrates, but susceptibility is unlikely among invertebrates, plants, and other lower organisms. Therefore, health risks posed by THRa active chemicals in humans are predicted to be shared among all vertebrates, including mammals, birds, reptiles, amphibians, and fishes. This case study demonstrates how SeqAPASS can be used toward the One Health objective of linking the health of humans, domesticated animals, wildlife, and plants and provides a rapid, cost effective method of species extrapolation toward this goal.

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A veterinary perspective on One Health in the Arctic

C. Sonne, Aarhus University AU Arctic Research Centre / Department of Bioscience; R.J. Letcher, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; B. Jenssen, Norwegian University of Science and Technology / Biology; J. Desforges, Aarhus University (AU) / bioscience; I. Eulaers, Aarhus University (AU), Arctic Research Centre / bioscience; E. Andersen-Ranberg, University of Copenhagen; K. Gustavson, Aarhus University; B. Styrishave, Dep. of Pharmaceutics & Analytical Chemistry; R. Dietz, Aarhus University

Exposure to long-range transported industrial chemicals, climate change and diseases is posing a risk to the overall health and populations of Arctic wildlife. Since local communities are relying on the same marine food web as marine mammals in the Arctic, it requires a One Health approach to understand the holistic ecosystem health including that of humans. Here we collect and identify gaps in the current knowledge of health in the Arctic and present the veterinary perspective of One Health and ecosystem dynamics. The review shows that exposure to persistent organic pollutants (POPs) is having multiple organ-system effects across taxa, including impacts on neuroendocrine disruption, immune suppression and decreased bone density among others. Furthermore, the warming Arctic climate is suspected to influence abiotic and biotic long-range transport and exposure pathways of contaminants to the Arctic resulting in increases in POP exposure of both wildlife and human populations. Exposure to vector-borne diseases and zoonoses may increase as well through range expansion and introduction of invasive species. It will be important in the future to investigate the effects of these multiple stressors on wildlife and local people to better predict the individual-level health risks. It is within this framework that One Health approaches offer promising opportunities to survey and pinpoint environmental changes that have effects on wildlife and human health.

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Applying a "One Health" Approach to the Assessment of PFAS: Opportunity to Prevent Unintended Consequences
T. Lopez, J.M. Diamond, Tetra Tech, Inc.; J. Flippin, Tetra Tech, Inc / Center for Ecological Sciences; M. Bowersox, S. Richards, Tetra Tech, Inc
The One Health concept recognizes the interconnected health of the environment, animals and humans and that a transdisciplinary approach can achieve optimal health outcomes by recognizing these interactions. Due to their ubiquitous presence from widespread usage in consumer products, commercial operations, and firefighting, and their environmental persistence, per- and polyfluoroalkylated substances (PFAS) are globally distributed in the environment and detectable levels of PFAS are present in virtually all humans, as well as terrestrial and aquatic animals. Using the One Health concept we evaluate PFAS in the environment through a review of toxicity information, fate and transport information, sampling data, and regulatory efforts. Based on environmental media and biota concentrations observed in our work and others at several sites around the globe, we illustrate likely exposure scenarios for biota and humans, and highlight some of the current challenges in assessing risks of this unique class of chemicals. Information from our research and that of others indicates that potential exposure pathways of PFAS in soils, sediments, and water to animals and plants are complete, and PFAS is transported through the food chain, presenting an additional exposure pathway to humans as well. Fish tissue PFAS data, for example, indicate potential linkages between ecological and human health suggesting that consumption of fish may be a major source of exposure to these chemicals. When combined with potential intakes through drinking water as well as consumer exposures through household items, the possibility exists for much higher intakes and much longer-term exposure than estimated through environmental media alone, or from consumer exposure alone. Using a One Health approach will help provide integrated assessments of PFAS. It will promote discussion of the efficiencies to the business community of a One Health approach to chemical exposure from manufacture and intended use, to environmental release and remediation.

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Lancet Commission on Pollution and Health: Findings, One Health Perspectives, and SETAC Opportunities
N. Basu, McGill University / Faculty of Agricultural and Environmental Sciences
Last year the Lancet Commission on Pollution and Health was released. As one of the report co-authors, here I discuss the findings of our work and also examine these through a One Health lens (e.g., parallel research on environmental quality). The Commission addressed the full human health economic costs of air, water, and soil pollution. Stark conclusions were drawn including, for example, pollution is responsible for 9 million premature deaths per year (3-times more than AIDS, malaria, and TB combined), more than 90% of these deaths are released in low- and middle-income countries, and that it may reduce the GDP of countries by up to 2%. Furthermore, these estimates (and many more), likely under-estimate the true costs of pollution owing to tremendous data gaps that remain. The Commission also pointed to several success stories that highlight that solutions and strategies can have co-benefits for the environment, health, and the economy. The Commission focused strictly on human health though similar questions can be asked about environmental quality. Here, as I systematically break down the Commission's report, I will take a One Health approach and provide examples from studies that are concerned with environmental quality and animal health (e.g., how many fish and wildlife prematurely die from exposure to pollution? What are the economic costs of this?). I will conclude with some recommendations for SETAC members based on our experiences with the Lancet Commission.

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Panel discussion

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Chemical Exposure via Plastics and Synthetic Rubbers: Quo Vadimus?

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Method for comparing the release potential of additives from plastic materials
S. Frattini, European Chemical Agency (ECHA); A. Ahrens, ECHA (European Chemicals Agency) / B4 Exposure and supply chain; H. Piha, European Chemical Agency ECHA; T. Alasuvanto, European Chemicals Agency
The release of hazardous substances from plastic materials is an area of high public interest. During the first two registration deadlines under REACH, several hundred substances potentially used as additives in plastics have been registered in volumes of 100 tonnes/year or more. However, for many of these substances, there is significant uncertainty on the hazards and potential releases from plastic matrices. In late 2016, ECHA and a number of Industry sector organisations have started a joint effort for characterising the uses of the various plastic additives and the corresponding potential for release from articles. This work aims to support: i) industry in improving the REACH registration information on the use of plastic additives and the related exposure potential, and ii) authorities in prioritisation and de-prioritisation of substances for initiation of regulatory processes. The project focuses on approaches and learnings when comparing and ranking the potential of additives to be released from plastic matrials: i) an overview of substances confirmed by industry as plastic additives; ii) development of a robust method to compare the release potential of additives from different plastic matrices; iii) grouping of additives by function and ranking according to their release potential. The presentation explains the method including uncertainties and limitations, and the outcome. Learning and potential follow up is discussed.

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Chemicals in plastic packaging: Prioritization case studies
K. Groh, B. Geueke, Food Packaging Forum Foundation; T. Backhaus, University of Gothenburg / Department of Biological and Environmental Sciences; B. Carney Almroth, University of Gothenburg Sweden / Department of Biology and Environmental Sciences; P. Inostroza, University of Gothenburg / Department of Biological and Environmental Science; A. Lennquist, Chemsec; D. Slunge, University of Gothenburg / Centre for Sustainable Development; L. Trasande, New York University School of Medicine; A.M. Warhurst, CHEM Trust; M. Maffini, Independent Consultant; J. Muncke, Food Packaging Forum Foundation / General Management
Around 40% of plastics produced globally are used to make packaging. Effective management of plastic packaging should consider not only physical hazards but also chemical safety aspects. Gathering information on the exact chemical composition of plastic packaging can support substitution efforts, but this task is not trivial, as plastic packaging can be made of multiple polymers with numerous additives, along with other components, e.g. adhesives or coatings. We compiled a database of Chemicals associated with Plastic Packaging (CPPdb), covering known chemicals used during manufacturing and/or present in final packaging articles. The current version (https://doi.org/10.5281/zenodo.1287773) lists 902 chemicals likely associated with plasic packaging and 3353 chemicals possibly associated. Based on harmonized health and environmental hazard classifications, 148 chemicals likely associated with plastic packaging were identified as very hazardous. However, the majority of CPPdb chemicals lacked any harmonized hazard information. To identify the most urgent substitution candidates, we performed two prioritization case studies focusing on human health and environmental effects. Importantly, prioritization is a decision tool strongly dependent on expert judgement, and different strategies could result in different priority lists. For environmental hazards, our prioritization steps considered information from ecotoxicological databases and scientific literature, and resulted in the selection of benzyl butyl phthalate (BBP, CAS 85-68-7) as the first candidate for a more detailed assessment. For human health hazards, our prioritization strategy considered biomonitoring data, endocrine disrupting properties, and regulatory requirements in Europe, and resulted in a priority list of five phthalates (BBP; dibutyl phthalate (DBP, CAS 84-74-2); diisobutyl phthalate (DiBP, CAS 84-69-5); bis(2-ethylhexyl) phthalate (DEHP, CAS 117-81-7); dicyclohexyl phthalate (DCHP; CAS 84-61-7)). Identifying and ranking hazardous chemicals associated with plastic packaging is challenged by the lack of transparency on the use and toxicity of numerous substances. Both prioritization case studies focussing on the environment and human health highlighted phthalates as substitution candidates. Despite various restrictions, phthalates are still found in plastic packaging worldwide. The exposure potential likely resulting from this use further justifies the need for targeted substitution efforts.

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Identification of chemicals in food packaging plastic films by non-targeted analysis
Y. Sapozhnikova, U.S. Food and Drug Administration / ARS; E. Hoh, San Diego State University / School of Public Health
Plastic food packaging is widely used for protecting food from microorganisms and extending its shelf-life, while also providing consumers with convenient food handling. However, known and unknown chemicals from food packaging materials may potentially migrate into packaged food and present a risk to consumers. Plastic stretch film, or cling film, is one of the most widely used food contact materials in commercial and consumer applications. It is also the most complex in structure and composition, constructed with multiple layers and chemical additives to improve the film properties, including antioxidants, light stabilizers, plasticizers, thermal stabilizers, lubricants, antistatic agents, *etc.* Other chemicals: non-intentionally added substances - chemical impurities, contaminants, and chemicals formed during degradation and/or reaction of added chemicals also may be present. The goal of this study was to perform identification of chemicals extracted from food packaging stretch films. The films were extracted with US FDA approved food simulant: water/ethanol, 5%/95%, plus with three organic solvents with different polarities: hexane, ethyl acetate, and acetonitrile, to cover both polar- and relatively non-polar chemicals. Non-targeted GC×GC/TOF-MS analysis based on >70% match similarity to the standard NIST mass spectral library and manual review of mass spectral matching was used for tentative identification. Overall, 64-91 compounds were tentatively

identified, and selective migration was observed for some compounds between different solvents/food simulants. Among these compounds, several classes of chemicals were identified: alkylated naphthalenes used as lubricants, polycyclic aromatic hydrocarbons (PAHs) including those on the US EPA priority pollutant list, plasticizers, polymer additives, UV filters, flavoring agents and fragrances, surfactants, adhesives, products of thermal degradation, and low chlorinated polychlorinated biphenyls (PCBs). The identified compounds were characterized based on use/source, chemical properties, and previously reported occurrences in plastic food packaging materials, food and/or the environment. Several chemicals found in plastic films in our study were not previously reported as migrants from plastic materials by previous studies. Future studies should focus on quantitative migration to estimate the amounts of migrating chemicals into food simulants and real foods to provide data for risk assessment.

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Wear and Tear of Tyres in the Global Environment: Size Distribution, Emission, Pathways and Health Effects
P.J. Kole, Open University / Science; A.J. Löhr, Open University; F.G. van Belleghem, Open University of The Netherlands; A.M. Ragas, Radbound University / Department of Environmental Science
Wear and tear from tyres significantly contributes to the flow of (micro-)plastics into the environment. Our study compiles the fragmented knowledge on tyre wear and tear characteristics, amounts of particles emitted, pathways in the environment, and the possible effects on humans. The estimated per capita emission ranges from 0.23 to 4.7 kg/year, with a global average of 0.80 kg/year. The emissions from car tyres (100%) are substantially higher than those of other sources of microplastics, e.g., airplane tyres (2%), artificial turf (12–50%), brake wear (8%) and road markings (5%). Emissions and pathways depend on local factors like road type and sewage systems. The contribution of tyre wear and tear to the total global amount of plastics ending up in oceans is estimated to be 5–10%. In air 3–7% of the particulate matter (PM2.5) is estimated to consist of tyre wear and tear, indicating that it may contribute to the global health burden of ambient air pollution which has been projected by the WHO at 4.2 million deaths in 2016 from which 77,550 in the USA and 1,028 in Finland. It is yet unkown how much tyre wear and tear contributes to this death toll, but if it contirubutes relative to its weight fraction, it would be reponsible for 130,000-300,000 annual deaths globally, from which 2,330–5,430 in the USA and 30–70 in Finland. The wear and tear also enters our food chain, but further research is needed to assess potential health risks. Tyre wear and tear remains in the environment for a long time. The degradation half-life in soil is estimated at 490 days and in sediment at 4900 days. Preventing tyre wear and tear from entering the environment on short hand seems infeasible as there currently is no alternative for synthetic rubber. Mitigation should therefore focus on emission reduction, i.e. the development of wear resistant tyres, self-driving cars (less wear), open asphalt concrete roads (capturing wear and tear) and improving sewers and waste water treatment plant efficiency. It is concluded that tyre wear and tear is a stealthy source of microplastics in our environment which can only be addressed effectively if awareness increases, knowledge gaps on quantities and effects are being closed, and creative technical solutions are being sought. This requires a global effort from all stakeholders; consumers, regulators, industry and researchers alike.

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Microplastics in the terrestrial environment - Impacts of tyre wear particles on soil animals
S.K. Selonen, Vrije Universiteit Amsterdam / Department of Ecological Science; L.N. Sackey, University of Chemistry and Technology / Department of Environmental Chemistry; R. Turja, Finnish Environment Institute, SYKE / Marine Research Centre; C. van Gestel, Vrije Universiteit Amsterdam / Ecological Science
Microplastics, i.e. plastic particles smaller than 5 mm and their potential effects in environment have raised growing public concerns. However, the research on microplastics has mostly been directed to aquatic environments, and their entering and possible effects in terrestrial food webs are poorly known. It has been estimated that tyre wear particles (TWP) are among the most abundant microplastic particles released to environment due to human activities. However, there is still a gap of knowledge on the fate and environmental concentrations as well as the effects of tyre wear particles especially in the terrestrial environment. To shed light on this subject, the effects of TWP with size range of 0-180 μm on the survival and reproduction of the enchytraeid worm *Enchytraeus crypticus* and the springtail *Folsomia candida* as well as survival, growth, reproduction and biochemical biomarkers of the earthworm *Eisenia andrei* were studied. Standard Lufa 2.2 soil (Lufa Speyer, Germany) and/or food were spiked with concentrations of 0.02 %, 0.06 %, 0.17 %, 0.5 % and 1.5 % of TWP in dry mass. Survival and reproduction of the springtail *F. candida* seemed to be affected by TWP, but as the response was not consistent in relation to the concentration in soil or the variation among the replicates, none of the treatments differed significantly from the control. Tyre wear particles in food did not affect enchytraeid worms, but when they were spiked in soil, reproduction slightly increased at the lowest concentration and slightly decreased at the highest concentration when compared to the control. There also seemed to be a slight negative trend in earthworm survival and a positive trend in the change of biomass with increasing TWP concentration, but no statistically significant differences between the treatments were found. The biomarker data have not been analysed yet. Our study shows that tyre wear particles can affect soil animals, but the effects are slight and not always consistently related to the increasing concentration of the particles. As tyre wear particles contain xenobiotics that can be harmful to biota, it is possible that these chemicals can accumulate in the terrestrial food web via ingestion of tyre wear particles by soil animals. This study is part of the project IMPASSE – Impacts of Microplastics in Agro Systems and Stream Environments.

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Poster spotlight

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State of the Science on Emerging and Novel Poly- and Perfluoroalkyl Substances (PFASs) (I)

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Environmental contamination and human exposure to PFAS near a fluorochemical production plant: Review of historic and current PFOA and GenX contamination in the Netherlands
W. Gebbink, PFA Brussels; S. van Leeuwen, RIKILT - Wageningen University & Research / RIKILT
Primary sources of per and polyfluprpalkyl substance (PFAS) emissions into the environment (water and air) are fluorochemical production plants (FPP), and once released into the environment, PFASs distribute to other abiotic media such as soil and sediment and accumulate into biota including in human food chains.A FPP located near the city of Dordrecht in the Netherlands has been producing fluoropolymers since the 1970s by using PFOA in this process until 2012 and GenX from 2013 onward. Over the last decade, peer-reviewed publications and reports have been published on the presence of PFOA in the Dutch environment (including surface, ground and drinking water, soil, vegetation and food), while publications and reports on GenX in these matrices were published mostly in recent years. The aim of this study was to review the contamination of the local environment near the FPP with PFOA and GenX relative to other locations in the Netherlands by using data for multiple environmental matrices published in the peer-reviewed literature as well as in the grey-literature. Besides environmental contamination, this review will also report on contamination of human exposure pathways (drinking water, food) with PFOA and/or GenX on a local and national level. Data were available on PFOA and GenX in surface water, ground water, soil and vegetation. These studies included multiple sampling sites nearby the FPP as well as other locations in the Netherlands. Data on PFOA and GenX in drinking water from numerous locations throughout the Netherlands was available as well as on both PFASs in fruit and vegetables.Preliminary analyses of the available data showed that PFOA has been monitored in surface water yearly since 2006 at several locations, while the earliest reporting of GenX was in 2013 followed by 2016 and 2017.Considerable higher GenX concentrations were found in downstream sampling locations from the FPP compared to other locations in the Netherlands, while this pattern was less clear for PFOA.PFOA and GenX were detected in drinking water mostly in the western part of the Netherlands where also the FPP is located. During the period of 2010 and 2016, PFOA concentrations ranged between 1.4 and 23 ng/L, while during 2016 and 2017, GenX concentrations ranged from 0.3 to 29 ng/L. Data review of PFOA and GenX in groundwater, soil, vegetation and food are ongoing.

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Evaluation of unknown precursors in paper-fibre biosolids contaminated sites by combination of sum parameters and PFAS analysis
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In the regions around Rastatt/Baden-Baden and Mannheim in southwest Germany ca. 600 ha of agricultural land is contaminated by per- and polyfluorinated alkyl substances (PFASs). This contamination was predominantly caused by the use of compost, mixed with paper-fibre biosolids, and applied to the fields, tentatively in the period 2000-2008. Besides perfluoroalkyl acids (PFAAs), other compound classes of grease-proofing agents in food-contact materials of paper and board, such as polyfluoroalkyl phosphate esters (PAPs), perfluoroalkane sulfonamidoethanol-based phosphate esters (SAmPAPs), perfluoroalkylpolyethers (PFPEs), and side-chain fluorinated polymers (poly(meth)acrylates, polyurethanes) are either proven or expected to occur in this area. These chemicals

can act as precursors to persistent and mobile PFAAs, which are transferred to groundwater aquifers and drinking water as well as to field crops. To assess the formation potential of mobile intermediates and persistent and mobile terminal products, we compared the results of target analysis for several PFAS classes to the sum parameters (i) extractable organic fluorine (*EOF*) in soil (ii) total oxidisable precursor (*TOP*) assay in soil and crops and (iii) to adsorbable organic fluorine (*AOF*) in aqueous soil leachates. In addition, non-target screening by LC-Q-TOF-MS was used to identify PFAS classes. In surface soils organofluorine levels up to the mg/kg range occurred. Organofluorine of PFASs from target analysis (ΣF(PFASs)) accounted for about 50 % of the EOF. (ΣF(PFASs) correlated well with the EOF in soil samples (r^2=0.99). Precursors were mainly located in the in the plough horizon (upper 40 cm). The maximum of the leachable, mobile fraction, measured as AOF in aqueous soil leachates was located about 10 cm below the plough horizon. Organofluorine of intermediates in soil accounted for 4.3–25 % of ΣF(PFASs). EtFOSAA was the prevailing intermediate with 0.4-18 % of ΣF(PFASs). Only short-chain PFAAs, PFOS, and traces of a few mobile intermediates were transferred to crops (wheat, maize, grass). Non-target screening revealed the occurrence of more than 100 PFASs in 18 homologous series. The precursors 6:2 to 12:2 (and mixed) diPAPs and their possible degradation products were the most abundant PFASs, followed by diSAmPAPs (8 homologues) and the degradation products EtFASAAs and PFOS. Polyfluoropolyethers (PFPEs) have been detected in several homologous series, but at low abundances.

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Emerging and legacy per- and polyfluoroalkyl substances in surface and deep waters from the North Sea to the Arctic

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Long-chain per- and polyfluoroalkyl substances (PFASs) have been shown to be persistent, bioaccumulative, toxic, and to undergo long-range transport. Consequently, an industrial shift has been taking place, moving from long-chain PFASs towards replacement substances, such as per- and polyfluoroether carboxylic and sulfonic acids (PFECAs and PFESAs). Due to structural similarities, the question arises whether the alternatives represent a substantial improvement. For legacy PFASs like PFOS and PFOA, it is known that they reach remote areas and accumulate there. However, experimental data on potential long-range transport of emerging PFASs is still limited. Modeling data suggest that several ether-based alternatives and their common predecessors possess comparable long-range transport potential. In addition, PFECAs and PFESAs have been detected in Europe, China and North America already. Together with first findings in Arctic biota, these data indicate that ether-based replacements can be globally distributed and are likely to undergo long-range transport. This study aims at investigating occurrence and distribution of emerging and legacy PFASs in surface and deep waters along potential transport pathways from the North Sea to the Arctic. 54 surface water samples and 6 vertical profiles from bottom to surface were taken during the *Polarstern* cruise PS114 in July 2018. The analytical method included 36 target analytes, among them the ether-based replacements GenX, ADONA and F-53 B. On board, 1 L water samples were spiked with 400 pg internal standards and loaded onto preconditioned solid phase extraction cartridges. After a washing step, the cartridges were dried under vacuum and stored at -20 °C. Further analysis was performed back at Helmholtz-Zentrum Geesthacht. After elution of the target compounds, eluates were reduced to 150 µL and [$^{13}C_8$]-PFOA was added as injection standard. Instrumental analysis was performed by HPLC-MS/MS. Concentration and composition profiles of PFASs will be compared considering the distance to emission areas, ocean currents and circulation, different water layers and potential correlations to salinity and temperature. In combination, this will give information on transport and sources of PFASs from the North Sea to the Arctic. Regarding GenX and other emerging PFASs, the results may provide additional evidence for long-range transport potential. This can be valuable to evaluate these compounds regarding future regulations.

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Spatial trends and tissue distribution of per- and polyfluoroalkyl substances, extractable organic fluorine and total fluorine in marine mammals

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Per- and polyfluoroalkyl substances (PFASs) are a diverse class of anthropogenic substances which display extreme persistence. Long-chain perfluoroalkyl acids are a particularly insidious class of PFAS due to their tendency to bioaccumulate and links to adverse effects in organisms. Despite numerous phase outs and international regulations, manufacture of alternative substances with similar physico-chemical properties continues. A recent report by the OECD listed more than 4700 PFASs-related CAS numbers, but the actual number of PFASs in global circulation may be higher. The objectives of this study were to address the structural diversity and vast number of PFASs accumulating in marine top predators using a multi-platform approach combining targeted PFASs analysis with suspect screening on HPLC-Orbitrap-MS and analysis of extractable organic fluorine (EOF) and total fluorine (TF) using combustion ion chromatography (CIC) to estimate the fraction of unidentified fluorinated substances. Liver samples from eleven different marine mammal species (polar bears, porpoises, dolphins, whales and seals) were collected from four locations (Sweden, Iceland, Greenland and USA Atlantic) to evaluate spatial trends. Additionally, nine different tissue samples (liver, lung, kidney, blood, serum, blubber, muscle, skin and ovary) of a killer whale were obtained to study the distribution of PFASs in a top predator. A total of 22 PFASs were detected over all species, with PFOS as dominant contaminant in most samples in concentrations of up to 1600 ng/g. PFUnDA was the dominant compound in humpback whale (Greenland), whereas the precursor 3-perfluoroheptyl propanoic acid (7:3 FTCA) dominated in harbor porpoise, harbour seal (US Atlantic) and grey seal (Iceland). Harbor porpoises from US and Sweden had comparably high Σ_{36}PFASs concentrations, while West Greenland porpoises had lower concentrations. In the killer whale, liver tissues contained the highest Σ_{36}PFASs concentrations (434 ng/g), accounting for 39% of all other tissues. Blood, lung, serum, kidney and ovaries had Σ_{36}PFASs concentrations of 100 up to 150 ng/g, while muscle, blubber and skin had comparably low Σ_{36}PFASs concentrations ranging from 12 to 32 ng/g. Overall, these data give an overview of the occurrence of legacy, emerging and unknown PFASs in marine mammals from the northern hemisphere including their spatial and tissue distribution.

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Toward a Comprehensive Emission Inventory of C4 to C10 Perfluoroalkanesulfonic Acids and Related Precursors: Focus on the Life Cycle of C6- and C10-Based Products

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Perfluoroalkanesulfonic acids (PFSAs) are an important class of per- and polyfluoroalkyl substances (PFASs). Of all PFSAs, perfluorooctanesulfonate (PFOS) and its precursors have been used most extensively. However, the C_6 and C_{10} homologues of PFOS, perfluorohexane- and perfluorodecanesulfonate (PFHxS and PFDS), were also used in many applications, often as surfactants or for surface protection. However, compared to PFOS, much less is known about their production volumes and uses. They have properties similar to those of PFOS and have also been released to the environment as shown by field measurements. Here we build on earlier work on compiling a global emission inventory for PFOS and use a similar approach to estimate global emissions of PFHxS and PFDS. The emission inventories for PFHxS and PFDS were calculated by integrating data about the production, use, and disposal of relevant chemicals collected and extrapolated from publicly available information with emission factors of different life-cycle stages. Included were PFHxS and PFDS, the sulfonyl fluorides PHxSF and PDSF, and the perfluorohexane and -decane sulfonamides and sulfonamidoethanols (*x*FHxSA/Es and *x*FDSA/Es). In a second step, the inventories were fed into a global environmental fate model, CliMoChem, to calculate environmental concentrations, which were then compared to measurements in seawater. A majority of the emissions are estimated to have occurred from production in the US, Western Europe and Japan before 3M's phase-out of POSF-based products in 2002 (113–864 t for PFHxS; 38–370 to for PFDS). Emissions of PFHxS are predicted to continue from product use and disposal (1–21 t) and degradation of *x*FHxSA/Es (1−66 t) between 2016 and 2030. The ranges of modeled concentrations include most of the field measuments. The modeled concentrations rise most rapidly in the northern temperate zone of the model, where most production occurred, and they reach a peak around 2002. More northern and southern zones show a delay before concentrations begin to rise after the start of production and also peak concentrations occur later. Elevated environmental concentrations of PHxSF, PDSF, and their derivatives are predicted to continue for decades to come, and zones farther from the source regions have not yet even reached their estimated peak concentrations. The favorable comparison with field concentrations indicates that our emission inventories are plausible, despite all uncertainties involved.

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Short-Chain Fluorotelomer-based PFAS: Recent Advances in Toxicology, Biodegradation, Water Remediation and Alternatives Assessment

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Per- and polyfluoroalkyl substances (PFAS) is a term that describes a wide and diverse array of chemistry containing fluorine and carbon. It is noteworthy that the chemistry and properties are vastly different across the various PFAS categories and classes. The primary focus of this presentation will be on the fluorotelomer-based products with six or less fluorinated carbons ("short chain") including fluorosurfactants, side-chain fluoropolymers and the potential metabolites and degradation products such as perfluorohexanoic acid (PFHxA). Fluorotelomer-based products can be in either the polymeric or non-polymeric PFAS categories. Within the non-polymeric PFAS category, fluorotelomer-based surface active agents (e.g. "fluorosurfactants") are used in complex multi-component formulations such as cleaning products, paints, coatings and Aqueous Film Forming Foams (AFFF). The non-polymeric fluorotelomer-based products provide superior surface wetting and leveling properties and unmatched AFFF fire-fighting performance on high-hazard Class B (i.e., hydrocarbon & polar solvent liquids) fires. Within the polymeric PFAS category are the side-chain fluorotelomer-based repellent products that are used in carpets, paper products and textiles providing essential and critical properties on high-end performance and medical garments, work wear, first responder gear and in military uses. The remarkable strength of the C-F bond provides products with superior resistance to extreme thermal, chemical and environmental conditions. This bond strength also makes them resistant to degradation and persistent in the environment. Industry best practices on handling and use will be reviewed. There have been significant advances in short-chain toxicology studies (development/reproduction; endocrine disruption), human health assessments, aerobic and anaerobic soil bio-degradation studies, progress in the removal of short-chain acids from ground and drinking water, and various other assessments. This presentation will summarize a number of these critical and significant advances and present the results in the context of the overall risk in using the short-chain fluorotelomer-based products. In addition, we will discuss decision criteria to help in the assessment of alternatives (risk vs. exposure), as well as challenges and success in the development of short-chain fluorotelomer-based products and an overview of their value-in-use including some critical end uses.

Contaminated Sediments: an Understudied Environmental Compartment (I)

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Automated SPME with reduced equilibration time for measuring freely dissolved concentration of hydrophobic organic chemicals in solid matrices
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Hydrophobic organic chemicals (HOCs) exhibit very strong sorption to the soil/sediment organic matter, and their water-organic carbon distribution coefficients (K_{OC}) are generally larger than 10^4-10^5 L/Kg. Freely dissolved concentration (C_{free}) of HOCs is then a more relevant measure than total concentration. C_{free} is a well-defined parameter for expressing chemical exposure and (bio)availability, since C_{free} quantifies the effective concentration for diffusion and partitioning, and to a large extend also for bioconcentration processes and toxicity. Although equilibrium sampling (e.g. by using Solid Phase Microextraction, (SPME)) is a powerful tool for determining C_{free} of HOCs, it generally requires too long equilibration times in the range of few days up to weeks or even months, virtually impossible to conjugate with time and cost-efficient methods and automated devices. Novel approaches to speedup sampling kinetics of HOCs are therefore needed. In the present study, two fully automated SPME methods for determining C_{free} of HOCs in sediment and soil samples were developed: 1) direct immersion (DI)-SPME at 35 °C and 2) headspace (HS)-SPME at 80 °C. The HS-SPME method was tested and cross-validated against the DI-SPME method to shorten equilibration time further. The assumption was that the matrix to SPME coating partition coefficients are temperature independent, which implies that the equilibrium concentrations in the fiber are the same for HS-SPME and DI-SPME. Passive dosing from preloaded silicone rods was used as calibration method. The two approaches yielded similar results thereby cross-validating each other. Sampling kinetics with HS-SPME at 80 °C were much faster compared to DI-SPME at 35 °C with equilibration times generally within 2-3 hours. Finally, PCB total concentrations (C_{total}) (including C_{free} and PCB associated to the solid matrix) were measured based on spiked 13C labelled PCB standards and K_{OC} values were determined as ratio of C_{total} over C_{free},normalized to the sample specific fraction of organic carbon. Data presented here showed that HS-SPME at elevated temperature is a new, practical and valid alternative to other passive sampling methods for determination of C_{free} of HOCs in solid matrices.

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Effects of simulated resuspension and amendment with activated carbon on metal and organic pollutant release from contaminated sediments
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Contaminated coastal sediments are a growing concern for ecosystem and human health. The harbor of Oskarshamn in south-east Sweden is heavily contaminated with As, Cu, Cd, Hg, Pb, Zn and the hydrophobic organic contaminants (HOCs) PAHs, PCBs, and dioxins. A remediation project has recently removed 400 000 m^3 of contaminated sediments from the inner harbour, however, the outer harbour is highly contaminated and would be logistically difficult to dredge. This study is part of the research project CAPTIVE (capping with reactive sorbents), where we evaluate the efficiency and applicability of remediation using reactive srobents *in situ* in the outer harbor of Oskarshamn. Amendment with activated carbon (AC) has been shown to effectively bind and reduce the bioavailability of HOCs in sediment. We present results from a laboratory experiment, where contaminant release from intact sediment cores of the outer harbour was studied with and without addition of a thin layer cap of either powdered (PAC; 15-35 μm) or granular AC (GAC; 425-850 μm) mixed together with clay. The AC dose (0.6 kg m^{-2}) was in the low range compared to other studies on thin layer capping with AC. We also tested the effects of particle resuspension on contaminant release by simulating resuspension using mechanical stirring. Results indicate that PAC amendment significantly reduced the release of PCBs and PAHs to overlying water. GAC amendment had no effect on PCB and PAH retention in static condition (no resuspension), but reduced the release of PCBs and PAHs in resuspended conditions. Resuspension led to an increased release of PCBs and PAHs from sediment. For metal cations, thin-layer capping with AC reduced the release of Cd, Co, Cu, U and Zn cations from sediments. No significant effect of AC capping was observed for Fe, Mn, Ni, and Pb. Resuspension generally decreased the release of Cd, Co, Mn and Zn, whereas Cu, Fe, Ni, Pb and U were not affected by resuspension. However, resuspension may cause increased transport of particle-bound contaminants from the harbour. The results motivate further research to find a suitable reactive cap material that may withstand resuspension and maintain a high retention efficiency over time without adverse effects on benthic organisms. *Pending results on sediment and pore water concentrations of HOCs*.

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Sorption of polycyclic aromatic hydrocarbons (PAHs) to biodegradable and conventional plastic materials in contaminated sediments
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Plastic debris is a growing threat in marine environments. The high abundancies of different-sized plastics on seafloors worldwide have led to suggest that these habitats could act as sinks for marine plastic debris. Being very resistant to degradation, plastics can cause long-term harm on seafloors for example by smothering and entangling organisms, or inhibiting gas exchange in the sediment-water interphase. It has also been observed that plastics can leach out harmful substances and adsorb environmental contaminants, such as polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from their surroundings; when ingested, plastic with their co-contaminants can pose threat to biota and food webs. To combat harm caused by persistent conventional plastics, biodegradable plastic materials have been proposed as a solution. However, there is currently very little knowledge on the degradation of biodegradable plastics in marine environments, and their ultimate fate and effects compared to their predecessors. Hence, a long-term laboratory experiment was conducted to study the sorption of PAHs from sediments and seawater to four types of conventional and biodegradable plastics; polystyrene (PS), polyamide (PA), cellulose acetate (CA) and poly-L-lactic acid (PLLA), and to study the microbial communities on these materials. Each plastic type was cut into different-shaped pieces with surface area of 1 cm^2 and 20 pieces of each plastic type were incubated in field-collected sediments or seawater for 14 weeks. Sediment and near-bottom water for the experiment were collected from two sites on the coast of Helsinki: West harbor, which was Europe's second most active passenger harbor in 2016, and Vanhankaupunginlahti, which is an enclosed bay that has been the recipient for municipal waste waters until the 1980's. Concentrations of selected PAHs were measured from the sediments before the experiment and from the plastics after the incubation period. The preliminary results indicate that there are differences in the sorption capacities of plastic types and measured contaminants in both of the studied matrices (sediment and seawater). These results emphasize the need to further study the properties and fate of biodegradable plastic materials in the marine environment to be better able to assess their potential risks to ecosystems.

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Depth-related analysis of sediment and pore water in microcosms
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Sediment toxicity testing of plant protection products (PPP) is gaining an increasing awareness within the scientific and regulatory community. Currently, PPP concentrations in sediment and pore-water of Chironomid toxicity studies acc. to OECD test guideline (TG) 218/219 are determined as mean over the entire sediment layer of the test system. Hence, a depth-related analysis can contribute to a more accurate assessment of the effective exposure for the predominantly surface sediment dwelling organism in these test designs. Therefore, a methodology was used enabling the depth-related analysis of test compounds in pore-water and adsorbed to sediment. After removing the water phase, plastic tubes were stung into the ca. 15 mm-thick sediment phase, which was subsequently frozen by liquid nitrogen. By the use of a cutting device, the sediment cores were cut into three slices providing a thickness of ca. 5 mm. Each sediment slice was centrifuged to isolate the pore-water. A sequential extraction was performed to determine the sediment adsorbed residues. After combining the sediment extracts, pore-waters and extracts were analysed by LC- MS/MS. Chironomid toxicity studies were and will be conducted acc. to OECD TG 218 and 219 applying four model compounds B (Koc≈ 300 L/kg), C (K_{oc}≈ 0 L/kg), D (K_{oc}≈ 3900 L/kg) and E (K_{oc}≈ 3400 L/kg) separately to the test systems. To investigate the spatio-temporal distribution of the compounds, test systems are incubated and processed at different days after treatment (DAT). After application to the water phase of the test systems (OECD TG 219), C was predominantly found in the overlying water phase and pore-waters of all sediment layers at 1-DAT. The amount of C measured in the top layer remained highest until 14-DAT. After 14-DAT, C concentrations levelled out until 28-DAT over all three layers due to low diffusion hindrance of this low adsorbing compound. Consistently, the mobility of C was proven after application to the sediment phase (OECD TG 218). One DAT, significant differences of the amounts of C could be measured in the pore-waters of the three-layer depths. From 3-DAT until the study end, the compound concentration gradient between the layers had balanced. These first results indicate that the used methodology can provide a substantial contribution to a more realistic determination of exposure concentrations in chronic water-sediment toxicity tests, opening the discussion for an improved sediment risk assessment.

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The bioavailability to fish of oil entrained in porous river bed sediments
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The expanding shipment of oil via pipeline and rail in Canada has increased the risk of spills to freshwater ecosystems. However, the fate of oil in freshwater is not well understood, particularly in west coast rivers where salmonids reproduce. These rivers are shallow and turbulent, with a high gradient, variable flow rates, and porous bed sediments derived from glacial till. In-stream pressure gradients create hyporheic flows through bed sediments between zones of down-welling and up-welling. Salmonids spawn in these zones because continuous flows of fresh water promote egg development until hatch (6-7 months). Hydraulic modelling demonstrates that hyporheic flows could entrain oil droplets into bed sediments, and model sediment columns readily trap oil between sediment particles. Water passing through columns of oil-contaminated sediment contains measurable concentrations of hydrocarbons that decline over weeks as the oil weathers by water washing. We assessed the potential exposure of salmonid embryos to oil in interstitial waters by measuring the bioavailability of dissolved hydrocarbons to juvenile trout exposed to effluents from columns of oiled sediments. Columns were loaded with a gradient of droplets of light and medium crude oil, heavy fuel oil, and diluted bitumen products. The amount of oil retained by the columns and the concentrations in water flowing through the columns increased with loading. The bioavailability of oil to trout, as indicated by induction of liver cytochrome P-4501A enzymes, increased with oil loading by up to 50-fold that of trout in effluent from non-oiled columns. Induction declined with time but persisted for at least 4 weeks. These experiments suggest that oil droplet trapping in bed sediments may occur following oil spills to rivers and pose a prolonged risk to the survival and development of salmonid eggs.

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Behavior and biochemical responses of the polychaete Hediste diversicolor to polystyrene nanoplastics
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Several stressors have been identified as potential key and/or emerging drivers of environmental change that may significantly influence marine near-shore ecosystems. Among these stressors, small plastic particles have been increasingly reported in aquatic environments. Although an increasing number of studies have been focusing in the effects of microplastics, the information concerning the effects of nanoplastics to marine organisms can be considered scarce, despite the increased potential to be incorporated by organisms. In the marine environment, nanoparticles are expected to agglomerate/aggregate, eventually settling and becoming available for benthic organisms. Nonetheless, the information concerning their toxicity impacts to organisms like polychaetes is scarce. Polychaetes are often the most abundant group of organisms in benthic communities and as they are in intimate contact with sediments, they are maximally exposed to harmful materials present there. Polystyrene plastics are among the most produced worldwide, for products with low reuse, making this polymer commonly found in the aquatic environment. Thus, this study aimed to assess the effects of waterborne polystyrene nanoplastics (100 nm) on burrowing activity and biochemical endpoints associated with oxidative status, neurotransmission and energy metabolism in Hediste diversicolor. Specimens of H. diversicolor were collected in a reference site of Ria de Aveiro lagoon (Portugal) and after acclimatization, 21 organisms per experimental condition were randomly distributed per 3 glass aquaria and were exposed for 28 d to different concentrations of 100 nm polystyrene particles (0.0; 0.005; 0.05; 0.5; 50.0 mg/L). Overall, exposure to polystyrene nanoplastics resulted in an impared burrowing behaviour in exposed polychaetes. Moreover, nanoplastics affected assessed biochemical endpoints like cholinesterase activity (marker of neurotoxicity) and electron transport (associated with metabolic activity). These results explain the observed altered behaviour. Nonetheless, no peroxidative damage was found suggesting that alterations in antioxidant defences prevent damage. The results obtained in this study indicate that tested conditions induce behaviour and biochemical alterations in the studied polychaetes. Considering that nanoparticles are expected to settle in the sediments, this study shows that these organisms may be a target of potential pernicious effects of nanoplastics.

Bridging the Gap: Maximizing the Role of Mechanistic Approaches (Including Omics) for Better Chemical Safety Decisions Across Humans and Ecosystems (I)

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High throughput metabolomics of single zebrafish embryos
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By substituting adult fish with embryos, the Fish Embryo Acute Toxicity (FET) test (OECD 236) has addressed some of the ethical and financial challenges associated with toxicity testing under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) legislation. Nevertheless, information obtained through the OECD 236 assay remains limited to conventional toxicological endpoints (e.g. LC_{50}, LOAEL, NOAEL), which provide little insight into a chemical's mode of action. With the ever increasing number of chemicals entering the European market, a mechanistic understanding of the adverse effects of these substances is clearly needed. This is highlighted by the introduction of the Adverse Outcome Pathway (AOP) program by the OECD in 2012. Metabolomics is among the Omic sciences which have shown considerable promise in generating data to support AOP development; however, pairing metabolomics assays with regulatory testing, and interpretation of metabolomic data within a regulatory context remains challenging. In this proof-of-principle study we present the application of metabolomics to single zebrafish embryos exposed to the beta-blocker propranolol according to the OECD 236 guideline. Incubation, microscopy, embryo extraction, and instrumental analysis were all performed in the same 96-well plate, resulting in a total assay time (i.e. testing of 6 doses of propranolol plus positive and negative controls, measurement of conventional endpoints as well as generation and multivariate analysis of metabolomics data from 96 samples) of < 12 days. Statistical analysis using a random forest repeated double cross-validation framework with unbiased variable selection (MUVR) revealed dose-specific perturbations of the metabolome, including at doses below the conventional lowest observed adverse effect level (LOAEL). By anchoring metabolomic effects observed at low dose to those at high dose (i.e. in the presence of an apical endpoint), results of this assay could potentially be interpreted within a regulatory context. Thus, in additional to rapidly generating both conventional and MOA-specific data suitable for regulatory applications, this assay could be more sensitive for detecting adverse effects at low doses.

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Fish hepatic proteome as a tool for environmental management of the safety limits of cadmium
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The aquatic environment constantly receives different contaminants, among them is the nonessential metal cadmium (Cd). This metal is largely used in industry, being present in products as batteries, pigments, and plastics. As other metals Cd can bioaccumulates in tissues of exposed organisms. In this study adult male of *Rhamdia quelen* were exposed to 0 (control), 1 and 10 $\mu g\ L^{-1}$ of Cd. After 15 days, animals were anesthetized in benzocaine and euthanized by medullar section, the liver was collected to proteomic analysis. Proteins were extracted in lysis buffer, run in SDS-PAGE, digested with trypsin and purified. Samples were submitted to liquid chromatography and quantified by mass spectrometry. Proteins were

identified by MaxQuant software using Siluriforms GenBank database. The results showed more than 1500 proteins expressed in liver of *R. quelen*. Among them, the histone 2B, an important protein in cell cycle, acting in the regulation of chromatin structure, was more expressed at 1 $\mu g\ L^{-1}$ of Cd. In the same group the β subunit of the protein Sec61, a protein translocation channel responsible to export the proteins throughout endoplasmic reticulum membrane, was less expressed. In the animals exposed to 10 $\mu g\ L^{-1}$ of Cd more proteins were differentially expressed. In this group, the myosin 9 increased and the myosin regulatory light chain 12B and myosin light polypeptide 6 decreased, both proteins involved with cytoskeletal activity. The expression of calmodulin, a calcium (Ca^{2+})-binding protein, therefore acting as mediator for the action of different Ca^{2+} dependent proteins and cellular processes, decreased at the higher Cd concentration. Some studies observed that Cd toxicity is related to Ca^{2+} homeostasis and the decrease in calmodulin is another evidence of this mechanism. Regarding to the endocrine system, the expression of the membrane-associated progesterone receptor component 2 decreased in fish exposed to 10 $\mu g\ L^{-1}$ of Cd. This receptor promotes a fast progesterone response, allowing a rapid modulation of the cell action in response to the activation of genomic signaling pathway. Although the concentrations tested are allowed in the Brazilian environmental legislation they altered the expression of important proteins. Thus, proteomics can be a tool to better understand the Cd mechanism of action and to improve the environmental management of health-supporting ecosystems such as freshwater resources and human safety limits in the legislation.

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A multi-omics approach for identifying and validating molecular key events using the Adverse Outcome Pathway framework

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The Adverse Outcome Pathway (AOP) framework is a concept developed in recent years to improve the risk assessment of chemicals. AOPs aim to compile existing knowledge about responses to toxicants at successive levels of biological organisation to improve predictions of adverse outcomes. Key events (KEs) within AOPs can be seen as deviations from a healthy state via a stress response that may be predictive of an AO and thus an indication of the potential health hazards of a chemical. We designed a three-phase experimental strategy, using transcriptomics, metabolomics and targeted assays to identify and validate molecular KEs associated with an AO relevant to chlorobenzene: non-polar narcosis. During phase 1 of the study, *Daphnia magna* were exposed to chlorobenzene at 0.032 and 0.1 mg/L at high temporal resolution (11 timepoints between 0 and 8 days, continuing to 21 days for phenotypic endpoint measures), to identify key timepoints at which significant molecular perturbations, potentially indicative of a chronic AO, could be observed. Based on this, we found that the highest number of significantly changing metabolites/lipids and differentially expressed genes between the control and chlorobenzene treatment groups occurred at 4 and 8 hours and at 6 and 7 days. During phase 2, we tested the reliability of our phase 1 observations at these four key timepoints. Using a new machine learning pipeline, putative KEs were mined from the ‘omics data. Candidate biomarkers, representative of putative KEs, were selected from subgroups of highly correlated genes and metabolites using sparse canonical correlation analysis. So far, this approach has identified 60 candidate genes thought to be involved in KEs relating to chlorobenzene exposure. In phase 3 (currently underway), the dose-response characteristics of the putative molecular KEs are being investigated to validate our discovered KEs, and to probe key event relationships (KERs). Overall our work demonstrates the potential of ‘omics technologies for filling knowledge gaps and for improving our understanding of chemical Mode or Mechanism of Action (MoA or MechoA), which will in turn allow more comprehensive risk assessments to be conducted.

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Cross omics effect assessment of co-exposure to plasticisers and metals on neurodevelopment

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This study was part of the largest project on the exposome globally, HEALS, which proposes the functional integration of cross–omics data to derive exposome-wide causal associations with adverse health outcomes. Untargeted metabolomics of 600 urine and plasma samples from cohorts running in Poland, Slovenia, Croatia, Greece and Italy, which studied the environmental causation of neurodevelopmental disorders in neonates and children across Europe. The aim of this study was to obtain mechanistic insight into how co-exposure to phthalates and heavy metals causes neurodevelopmental dysregulation coupling human data with in vitro assays. Specifically, HepaRG cells were exposed to mixtures of DEHP, DiNP, and BBzP phthalates, methylmercury and total mercury. These were the most abundant pollutants in the human cohorts. The effective in vitro concentrations of the chemicals were estimated through estimation from human biomonitoring data using internal dosimetry modeling on the INTEGRA computational platform. High dimension pathway analysis of transcriptomics and proteomics data revealed that co- exposure to phthalates and metals leads to dysregulation of the urea cycle due to alterations in the expression levels of arginase-1 and -2, argininosuccinate synthase, carbamoyl-phosphate synthase, ornithine carbamoyltransferase, and argininosuccinate lyase. Co-mapping proteomics and metabolomics data showed that their common drivers are responsible for the allostasis of metabolic pathways related to choline, phosphatidylcholine, phospholipases and triacylglycerol metabolism. The identification of the urea, phosphatidylcholine biosynthesis I and phospholipases metabolic pathways is of particular interest since these pathways have been also identified in human samples from the studied cohorts using untargeted metabolomics analysis as being associated with impaired psychomotor development in children at the age of three to six. Our work reveals that co-exposure to plasticizers and metals disturbs biochemical processes related to mitochondrial respiration during critical developmental stages that are clinically linked to neurodevelopmental perturbations.

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Systems biology approach reveals the effects of exposure to urban environment

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Introduction. Vast amounts of data have been and are generated for understanding various environmental stresses, ranging from most controlled laboratory exposures to field studies. It is also important to understand the exposure effects to humans, but for ethical reasons, these must be studied differently. Human exposomics studies have previously used personal devices, occupational and living area information for the study participants. Aim. Our aim was to explore the use omics data from a population-based biobank to understand the effects of exposure to urban life. Estonian Biobank currently has 52000 people genotyped and other omics data is available for some individuals. For example, 10840 individuals have ^{1}H-NMR metabolomics, 917 Illumina BeadChip gene expression data and 442 Olink proteomics data available. In addition, for each individual, questionnaire data and data from electronic health records are also available. Results. Our results show that there are 717 genes differentially expressed between city-born and rural-born people and 2319 genes differentially expressed between town-born and rural-born people. The main differences are between people born in urban areas as compared to rural areas. Specifically, genes differentially expressed between urban and rural areas are enriched in Gene Ontology terms such as “metabolic process”, “gene expression”, “organic cyclic compound binding” and “regulation of response to stress”. To further understand the large set of differentially expressed genes, we created a mutual information network. This network was modularized and functionally annotated, revealing modules enriched in various biological functions and diseases. For example, one module which is enriched in Gene Ontology term “response to stimulus”, is also enriched in brain disease and nervous system diseases. Other significant Gene Ontology terms include “Response to stress”, “translation”, “gene expression”, “DNA damage response, signal transduction by p53 class mediator”. Enriched diseases include immune system diseases, neoplasms, metabolic diseases and liver diseases. Conclusion. Our results have shown that systems biology analysis of omics data from a population-based biobank analysed with systems biology methods provide meaningful results to be explored further.

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Environmental contaminants modulate the transcriptional activity of polar bear (Ursus maritimus) and human peroxisome proliferator-activated receptor alpha (PPARA)

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Species at the top of Arctic marine food webs, such as the polar bear (*Ursus maritimus*), accumulate high levels of organohalogen contaminants (OHCs) including polychlorinated biphenyls (PCBs), organochlorine pesticides, and perfluoroalkyl substances (PFASs). Recent correlative field and *in vitro* studies indicate that contaminants also target polar bear lipid metabolism. Contaminant-mediated adverse effects towards energy metabolism is of particular concern in polar bears due to global climate change. Exposure to environmental contaminants and their potential effects towards energy metabolism is also of high concern in human populations. Exposure to PFASs and other environmental contaminants has been linked to metabolic disorders in animal models and human epidemiological studies. Peroxisome proliferator-activated receptor alfa (PPARA/NR1C1) is a ligand activated nuclear receptor that is a key regulator of lipid metabolism in tissues with high fatty acid catabolism such as the liver. However, PPARA-ligand interactions are likely species-specific, because sequences of ligand binding domains (LBD) of nuclear receptors vary between taxa. Understanding species-specific receptor-ligand interactions is particularly important for risk assessment. Here, we cloned PPARA from polar bear liver tissue and studied *in vitro* transactivation of polar bear and human PPARA by environmental contaminants using a luciferase reporter assay. Six hinge and ligand-binding domain amino acids have been substituted in polar bear PPARA compared to human PPARA. Perfluorocarboxylic acids (PFCA) and perfluorosulfonic acids induced the transcriptional activity of both human and polar bear PPARA. The most abundant PFCA in polar bear tissue, perfluorononanoate, increased polar bear PPARA-mediated luciferase activity to a level comparable to that of the potent PPARA agonist WY-14643 (~8-fold, 25 μM). Several brominated flame retardants were weak agonists of human and polar bear PPARA. While single exposures to polychlorinated biphenyls did not, or only slightly, increase the transcriptional activity of PPARA, a technical mixture of PCBs (Arochlor 1254) strongly induced the transcriptional activity of human (~8-fold) and polar bear PPARA (~22-fold). Polar bear PPARA was both quantitatively and qualitatively more susceptible than human PPARA to transactivation by less lipophilic compounds.

New Frontiers in Life Cycle Inventory Data Collection and Modelling (I)

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Combining Computable General Equilibrium Model and Life Cycle Assessment for Carbon Footprint Projections in Japan
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Since 2015, Science Based Targets (SBT) have been paid attention in the world. The targets adopted by companies reducing GHG emission to keep global temperature below 2 degrees increase from that of preindustrial revolution. Approximately 440 companies in the world declared to follow the targets. The targets for the mitigation are based on the calculated results of Integrated Assessment Models (IAM) such as Asia-Pacific Integrated Model (AIM). However, these models usually do not consider the entire supply chain, because of the differences of the aims of application. In contrast, Life Cycle Assessment (LCA) considers the entire supply chain. On the other hand, most of the LCA studies use the current environmental data and normally do not include the dynamism of system (i.e. they are not temporally differentiated). Therefore, this study aims at the development of a dynamic evaluation of environmental burdenbased on life cycle thinking to use both advantages of IAM and LCA. We tried to develop the database using AIM/CGE [Japan] developed by the National Institute for Environmental Studies (NIES). In this study, we collect the fundamental data using LCA databases and estimate GHG emissions in the future considering the supply chain among industrial sectors. We estimated the GHG emissions in 2005 as a tentative result. The total emission is approximately 1.60Gt-CO2eq. We confirmed the validity compared with the existing report published by the Japanese ministry of environment. We estimated the environmental impact projection in the future considering scenario like Shared Socioeconomic Pathways (SSP).

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Direct sampling to improve accuracy of Monte Carlo uncertainty analysis
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Standard Monte Carlo sampling in LCA uses independent sampling of parameter values. Independent sampling is computationally simple, but neglecting correlation between inventory parameters leads to confusing or even impossible results. We present a new, simple, and powerful approach that allows for the inclusion of parameter correlations and avoids fitting errors through the standardized provision of pre-calculated sample values for certain parameters. We have implemented this approach in an open-source library called Presamples, which builds on top of the Brightway open-source LCA software framework.\n\nMost LCA software and databases allow for the use of parameterized datasets – datasets where certain parameter values are defined by variables and formulas. Similarly, such data can be generated by external process models. Our approach allows for parameterized sample values to be generated beforehand and used during sensitivity analysis. These values can be generated by authoritative sources and tested for validity and randomness.\n\nGlobal sensitivity analysis often requires specific sampling strategies, which are difficult to implement with conventional LCA software. By moving the generation and filtering of Monte Carlo samples outside the Monte Carlo iterative calculation, pre-calculated samples allow for the easier generation, validation, and exchange of such values.\n\nThere is a high degree of both spatial autocorrelation and correlation due to model uncertainty in regionalized CFs. Accurate representations of such uncertainty require CFs to be supplied directly by the LCIA model and used directly.\n\nIndependent sampling breaks mass, energetic, or other balances, as inputs are not linked to outputs. Similarly, market datasets should sum to a fixed value, usually one - a constraint that is broken by independent sampling. Pre-calculated samples allow such balances to be explicitly included, and allow for the use of measured data in market shares.\n\nWe illustrate our approach using case studies of parameterized datasets in ecoinvent, European electricity generation and trade, and regionalized land use.\n\nThe use of pre-calculated sample values presents significant potential to improve the realism of LCA uncertainty analysis results in many application scenarios. Our open-source software and standardized data format could form the foundation for the adoption of such pre-calculated samples throughout the LCA community.

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Dynamic LCA modelling for metals production
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Life cycle assessment (LCA) is widely used in environmental assessment, but its application in the mining and mineral sector is still in progress. The value chain of metals production combines vast amounts of information from disciplines such as geology, mining, metallurgy, technology, environment and economics, all having a link to the environmental impacts of the mine project. Furthermore, the data from these processes is spatially and temporally restricted. Advances in computing power and simulation software enable various data sets to be incorporated in a single digital platform enabling more efficient and transparent data sharing. In addition, linked data sets and simulation can support assessment and optimization of the project as a whole rather than focusing on optimization of individual events. The current study proposes an advanced option of collecting process data and adding dynamic capabilities to a continuous LCA-based process optimisation. It combines metallurgical process simulation with a standard life cycle approach (cradle-to-gate assessment) and allows the usage of real-time monitoring data (e.g. daily sequence) as primary data from the process. It also considers that the metallurgical balance is consistently correct over time. Moreover, it allows a prediction of the KPIs by using process simulation. The results from estimating the environmental impacts based on a dynamic, one-month data set of the underlying metals production process clearly indicates volatility in all the monitored KPIs and shows the impact of changing ore grades and plant capacities on the carbon emissions of the process. In this case, the total emissions will increase when the feed rate decreases, despite of an environmentally favorable effect of the increased recovery of the concentrator process. This is because the subsequent hydrometallurgical processing plant is most environmentally efficient with the highest possible production rate. In sum, instead of taking metal production as a black-box and its environmental impacts as a constant, LCA practitioners should keep in mind that that kind of non-dynamic approach is not able to adequately address site-specific impacts to capture the spatial and temporal dimension of the results. This dynamic model consisting of an advanced process simulation model linked to LCA will be able to produce reliable LCI data as well as predict and optimise the environmental impacts based on changing process conditions and ore types.

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A bottom-up approach to build life cycle assessment models using agent-based models: the case of hydrogen supply chain for mobility
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Hydrogen has recently sparked a new research interest in hydrogen based mobility because of its potential as a means to store surplus energy from renewable sources for example. Hydrogen based mobility is currently only applied marginally in local or regional contexts, thus rendering the evaluation of environmental impacts of the hydrogen supply chains a challenging task. To investigate the claims regarding the environmental impacts of hydrogen from renewable energy sources we propose an agent-based modelling (ABM) framework to model the supply chain, feeding data to the inventory of a life cycle assessment (LCA) study. As an initial prototype of the framework, we have applied it to the greater region of Luxembourg. The objective of the study is to model how to satisfy the demand for hydrogen by the mobility sector, while minimizing the total hydrogen supply cost. The novelty of the approach does not rely on the use of ABM solely, but on the use of *emerging supply chains* from the ABM. With these supply chains as output of the ABM, the life cycle inventory (LCI) processes describing hydrogen production, storage and transport are connected among each other. The entire supply chain in turn is connected to the existing LCA process database. Finally the environmental impacts per functional unit (i.e., 1 kg of hydrogen at the pump) can be assessed. Preliminary results indicate that hydrogen from these emerging supply chains has a lower global warming potential compared to hydrogen from the existing market. During the presentation, the specific ABM implementation

details (Agent characterization, behaviours and environment), as well as simulation results will be used to illustrate how we derived the life cycle inventories and the actual life cycle assessment as well as the impact assessment.

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Enhancing data creation and adaption to different user needs: approaches in managing a large LCI database
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LCI data are time-consuming to collect and process, and data gathering is often a major time factor for LCA practitioners. Innovative data handling and processing can save key resources for data gatherers, providers and users. The architecture of the ecoinvent database is becoming more adaptable, flexible and efficient for this purpose, at all levels of interaction. On the data entry side, ecoinvent has boosted the possibility of generating easy and high-quality data, by developing LCI creation support tools in different sectors such as waste and wastewater treatment, crop production, and petroleum refinery operation. Open to data providers, ecoinvent has fine-tuned its submission and review platform with the years, making it ready to manage even larger submissions more effectively. At the same time, ecoinvent also collaborates with databases that operate on ecoinvent' s background data but remain external. ecoinvent has been using a modular approach to generate different system models, based on the same peer-reviewed free-of-access unit processes, that represent multifunctional and independent inventories. Today, new features allow the creation of hybrid system models, where two distinct databases are linked together and can serve multiple purposes at the same time, to enhance the power of choice of the user. It is the case of the chemical database delivered for the EU Product Environmental Footprint (PEF), pilot phase, as well as for a hybrid database constructed to suit the needs on Swiss consumption modeling of the Swiss Federal Offices. We will describe these modulating features, that give even more flexibility to the calculation and use of the database and increase the usefulness that practitioners can draw from the same data.

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Poster spotlight

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The Indoor Environment: Emerging Contaminant Identification, Analysis and Quantification for Exposure Assessment

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A coupled model of heat and chemical mass balance to consistently evaluate human exposure in indoor environments
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New building design and renovation standards tend to increase airtightness and use of newer materials to meet current environmental challenges. This leads to particularly confined spaces in which a wide variety of chemicals are released from materials and surfaces and expose the occupants to multiple health hazards. Learning how to design energy efficient buildings with good indoor air quality is one of tomorrow's challenge. An important source of indoor air pollution arises from the chemicals embedded in the building materials that are released in the indoor air of the dwelling and whose physicochemical properties are thermally driven. Temperature in building materials is commonly considered as the same as the room temperature, affecting substantially chemicals diffusion and offgassing. Once released in indoor environments, their residence time is impacted by physical removals such as ventilation. However, the impact of most of the design choices on chemicals fate remains unclear. Therefore, there is an urgent need to address chemicals fate together with heat transfers. We propose a new approach to consistently evaluate the impact of early design choices on human exposure in indoor environments as well as on energy consumption by coupling heat and mass balance. A set of ordinary differential equations (ODE) is obtained to formulate the conservation laws of heat and mass in the gas and in the solid phase. Occupants exposure during building use stage is characterized by the Product intake Fraction (PiF) for each exposure pathway, used to comparison of the relative importance of these pathways, as well as the potential exposure between chemicals or building designs. Coupling heat and chemical mass transfers in the building envelope enables a better understanding of the chemicals fate in building materials. The model, implemented on a low-energy dwelling, demonstrates that an air renewal rate of 0.6 1/h with a heat exchanger was the best trade-off to reduce energy consumption while ensuring a satisfactory air quality and limiting exposure to off-gassed chemicals. This approach paves the way to guide early building design choices towards a better indoor air quality without burden shifting on the energy balance.

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Indoor Dust/Air Partitioning: Evidence for Kinetic Delay for Low-Volatility SVOCs
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Dust-air partitioning ratios were estimated using COSMO-RS solvation theory, using the "liquid oligomer" approach to represent dust as a solvation medium. Using a variety of oligomeric models based on typical constituents of household dust, $K_{Dust-Air}$ value (g^{-1}/m^{-3}) were estimated and. Oligomeric models based on the structure of polymeric nylon-66, lignin, cellulose, and two protein strands representing head and body hair keratin. Experimental data was obtained from three published sources: Venier et al., 2016 (BFRs in indoor air and settled dust; Tay et al., 2017 (flame retardants, PBDEs and hexabromocyclododecanes in house dust); , Luongo & Ostman, 2015 (organophosphate and phthalate ester in settled and airborne dust). The modelling results for the three data sets combined clearly show a lack of strong correlation between the estimated and experimental partition ratios ($R^2 = 0.65$). The deviation is greatest for relatively low vapor pressure molecular species. This observation suggests that the disparity is due to a "kinetic delay" associated with oleophilic substances, wherein chemicals with very low vapor pressures require relatively long times to reach equilibrium with surrounding air, resulting in measurements of log K(dust-air) higher than equilibrium values. The model improved steadily as the chemical species in question were eliminated in order of increasing vapor pressure, up to a cut-off of about VP = 10^{-5} Pa. With this change, *n*-octanol and cholesterol yielded the lowest RMSE and highest R^2, with n-octanol marginally outperforming cholesterol. The remaining models ranked according to diminishing hydrophobicity. The onset of kinetic delay at about 10^{-5} Pa indicates a value which could be considered to be a lower-limit for SVOC definition in indoor air contexts. A significant implication of this result is that indoor dust concentrations of such low vapour pressure SVOCs should not be used as an indirect measure of indoor air concentrations of these chemicals, since they will not be expected to be at equilibrium with the air. For such chemicals, calculated air concentrations will generally be consistently and often significantly higher than in reality, due to air turnover losses that occur much faster than the time to equilibration.

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Do we need to re-think processes of chemical movement indoors?
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Modern indoor environments contain numerous natural and synthetic chemicals held in consumer products, furnishings and building materials. People have typically not been considered in mass balance models of indoor chemical fate or in the case of the model of Zhang et al. (2014) treated as another compartment. In these models, chemical transport is described in terms of the physics of gas- and particle-phase compounds. Human activities can be important, such as cleaning, as a chemical removal process. However, consideration of those activities is limited. What if people's "everyday" activities directly influenced the transport of chemicals within the indoor environment? We conducted a study of 51 homes and their residents in Ontario, Canada. The study involved collecting information about the homes and residents, and chemical concentrations in air, floor dust, and wipes of surfaces of electronic devices and participants' hands. We analyzed 23 organophosphate esters (OPEs), 14 polybrominated diphenyl ether (PBDEs) congeners, 10 non-PBDE halogenated flame retardants (HFRs) and 6 phthalate esters. Detection frequencies were >80% for 5-8 OPEs, 3 PBDEs, 2 non-PBDE HFRs and all 6 phthalates. Almost all surfaces, namely electronic surfaces and hands (backs and palms), had similar chemical profiles contrary to the expectation of electronic surfaces being dominated by one or only a few flame retardants which was only the case for TVs. Thus, hand surfaces had indistinguishable chemical profiles relative to electronic surfaces, especially handheld electronic devices. The handheld devices had higher concentrations of all analytes than non-handheld devices, with the exception of DBDPE and BDE-209 that had elevated concentrations in TVs. We constructed chemical profile networks and analyzed their structure to investigate the relationship between chemical profiles on surfaces. Hands were central to the networks of OPEs, HFRs and phthalates. After hands, air was central to the networks of lower molecular weight compounds (e.g., phthalates) and dust was central to the networks of higher molecular weight compounds, (e.g., PBDEs). We interpretted these results to indicate that hands transport chemicals from surface to surface, leading to non-unique chemical profiles on all surfaces sampled. This notion—of hands as agents of chemical transport—is consistent with the role played by hands touching surfaces as the mechanism of disease transmission.

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Concentrations of BFRs, PFRs and PFASs in Indoor Air and Dust from Four European Cities

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Introduction Contact with indoor air and dust containing brominated flame retardants (BFRs), organophosphate flame retardants (PFRs), and perfluoroalkyl substances (PFASs) is an important human exposure pathway. This study tests the hypothesis that recent restrictions on such chemicals has led to reductions in their concentrations in indoor environments and iincreased levels of substitute chemicals. **Sampling and Analysis** Between December 2016 and February 2017, samples of indoor floor dust were taken from homes in: Amsterdam, Netherlands (n=11), Antwerp, Belgium (n=20), and Galway, Ireland (n=10). Dust samples were also collected from offices in Stockholm, Sweden (n=10) and Amsterdam (n=10). Indoor air samples were collected from the same 10 Galway homes and Stockholm offices. PBDEs, HBCDDs, TBBP-A, and a range of emerging BFRs (EFRs) and PFRs were determined in Galway air samples and in all dust samples, with the exception of those from Amsterdam for which only EFR data are available. In addition, concentrations of a number of PFASs were determined in dust from homes in Amsterdam (n=9), Antwerp (n=6), and Galway (n=10), dust from offices in Amsterdam (n=9), and Stockholm (n=10), as well as in air from Galway homes and Stockholm offices. PBDEs, EBFRs, and PFRs were determined using GC-MS, with LC-MS used to measure HBCDDs, TBBP-A, and PFAS. **Conclusions** Our results confirm the ubiquitous presence of BFRs, PFRs, and PFASs in European homes and offices and the continued existence of substantial differences in contamination levels and patterns between different EU states. Median concentrations of BDE-209, HBCDD, and DBDPE are markedly higher in Galway house dust than elsewhere. This may stem from Ireland applying flame retardancy regulations to soft furnishings that are similar to those in the UK, and differ to those applied in other EU countries. PFRs predominate in samples from Antwerp, Amsterdam, and Stockholm, and are at a similar level to BDE-209, HBCDD, and DBDPE in Galway. Offices in Amsterdam display the highest median concentrations of PFOA, PFOS, PFHxS, and PFBS. PFBS predominates amongst these PFAS in all but Stockholm office dust. This may indicate increased use of this short chain PFAS (and/or its precursors) in response to restrictions on PFOS and PFHxS. Likewise, the elevated DBDPE concentrations in Galway homes implies its increased use in response to restrictions on the use of Deca-BDE of which BDE-209 is the major component.

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Levels of legacy and novel brominated Flame Retardants (BFRs) in Irish Human milk: Results from the Irish ELEVATE project 2016 - 2018

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This project aims to measure levels of flame retardants (FR) hexabromocyclododecane (HBCDD), polybrominated diphenyl ethers (PBDEs) and decabromodiphenyl ethane (DBDPE) in pooled samples of human breast milk from 92 Irish primiparas. Concerns regarding the impact of these chemicals on animal and human health eventually led to their classification as POPs, PBDEs and HBCDD have been listed as persistent organic pollutants (POPs) under the UNEP Stockholm Convention.Following bans and use restrictions for HBCDD and PBDEs, market demand resulted in manufacture of replacement FRs such as DBDPE. Analysis of human breast milk can provide a direct measurement of the human boody burden of POPs. The Food Safety Authority of Ireland performed a national survey on POPs in breast milk in 2013. The objective of this study is to replicate as far as possible the sampling strategy of this earlier Irish Biomonitoring study, and compare results to those of other European countries as well as previous Irish studies, to examine the evidence for possible temporal reductions in human body burdens in Ireland in response to actions (both legislative and voluntary) designed to reduce human exposure over the last decade. The sampling strategy was developed in line with that of the previous Irish bio-monitoring programme for POPs, and (with minor deviations) the 4thWHO UNEP protocols. Primiparas were recruited from the University Hospital Galway located in the west of Ireland and the Coombe Women's & Infants University Hospital located in Dublin between 2016–2018. Samples (30 - 60 mL) of breast milk were procured from participating primiparas and stored at -20 °C prior to pooling. Aliquots of pooled samples were extracted into 100 mL of dichloromethane (DCM) using pressurized liquid extraction (PLE) and subsequently cleaned by passing extracts through acid silica–packed columns. Samples were analysed via LC-MS/MS and GC/MS. Significant decreases are observed in concentrations of the PBDE congeners BDE-47 and BDE-153 ($p<0.01$,$p<0.05$) and some congeners are not detected (BDE-99 and BDE-100) when compared to results from the last Irish HBM in 2010, most likley reflecting the impact of voluntary and legislative bans on the use of these flame retardant containing products. Exposure estimates for nursing infant exposure to BDE-209 and BDE-47, were calculated following US EPA guidelines, and are below USEPA reference daily dose comparison guidelines.

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Biomonitoring of indoor air toxins with C. elegans

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Indoor air problems in schools and other public buildings weaken the health, well-being and working capacity of schoolchildren, their teachers and other exposed people. The aim of our project has been to develop biological tests based on the *C. elegans* nematodes, with which we could easily, reliably and reproducibly monitor overall toxicity caused by chemicals or microbial toxins, especially those that are present in the indoor air of moisture-damaged buildings. The nematodes have a well-developed chemosensory system, which helps them to find food and avoid pathogens. We have used transgenic nematode strains carrying fluorescent reporters driven by stress-responsive promoters and shown that exposure of these animals to indoor air toxins results in dose- and time-dependent fluorescent responses that can be quantitated by spectrometry or microscopy. By imaging we can evaluate the effects of toxins on fertility, motility and mortality of the animals and thereby distinguish between lethal and sublethal responses. Furthermore, since the *C. elegans* nematodes are transluscent, their imaging may give initial hints on whether the toxins have general effects or more specifically target the respiratory system, the nervous system or the immune system. Thus, as full organisms the nematodes provide several additional benefits as compared to other toxicity tests based on bacterial or eukaryotic cell populations, but further studies are still needed to validate their ability to distinguish between healthy and harmful buildings also under field conditions.

New Developments in the Science of vPvB and PBT Assessment (I)

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What are currently the most important topics for the identification of PBT/vPvB substances in the view of the regulators?

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The PBT Expert Group, having representatives from the EU Member State Competent Authorities (MSCA) the Commission, ECHA, industry and NGOs provides advice to PBT/vPvB assessments, updates the PBT/vPvT assessment approach as provided in ECHA Guidance and provides coordination and advice to the work on specific approach development topics, which have been identified by the group. In the autumn of 2018 a prioritisation activity for the current approach development topics of the PBT EG was undertaken. A total of 32 topics were scored in the online survey tool Webropol and 18 PBT EG members (out of a total 33) answered the survey. The scoring was based on perceived importance in terms of supporting and making the PBT/vPvB assessments more effective and on estimated human resource need and time for undertalking the work for the topic. The prioritisation activity resulted in the topics being listed according to importance and 9 topcis were assessed as being of high importance. The majority of the high importance topics are related to the bioaccumulation (B) assessment and they concern alternative means and methods for indicate bioaccumlulation potential for particular substance groups, where a comparison to the BCF criteria may not be the most representative way. It is perceived that there is a need to define other indicators, criteria and threshold values for the B assessment than the current ones of BCF and logKow. Other currently important topics concern clarifying the concept of weight-of-evidence and benchmarking in the PBT assessements and setting standards for these. The relatively new topic of practical expectations and limitations of chemical analysis in environmental fate testing for

REACH also gained a high score, as did the topic of specifying the issue of non-extractable residues (NER) in the guidance. A shift in focus from P- (persistence) to B endpoints has been an observed and the priority setting of the PBT EG reflects changes in priorities and experience gained over time. As the majority of the seats in ECHA's PBT EG are held by MSCA and other authorities, the priority setting of the approach development topics can be seen as reflecting the current focus on PBT issues by regulators. This indication may be of value for various stakeholders concerned with PBT issues and it may encourage increased collaboration on the developments that are currently believed to improving the identification of PBT properties in substances in the future.

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Effects of adaptation of WWTP sludge on the biodegradation of emerging pollutants

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Regulatory determination of the persistency of organic chemicals is often based on the results of OECD ready biodegradability tests (RBTs). These tests, however, suffer from several problems that lead to a high variability of the results and, hence, to difficulties in their interpretation. The origin and history of the inocula is one of the major causes of that variability. Nowadays, it is evident that results of RBTs change over time as microbial populations apparently adapt to metabolise previously persistent chemicals. As such, there is a need to assess the influence of this process on biodegradability tests. In this study we assessed the extent of the inocula origin influence on the outcome of biodegradability testing. For this, we compared the biodegradation capacity of five different wastewater treatment plants across the Netherlands to eliminate carbamazepine, n-methylpiperazine, metformin, diclofenac and 4-chloroaniline. Most of these products can be considered as emerging pollutants and are environmentally persistent. In a second time, we used chemostat systems to expose activated sludge microbial communities to carbamazepine, n-methylpiperazine, metformin and 4-chloroaniline for several months under defined conditions. In both experiment, the biodegradation capacities of the inocula were assessed in biodegradation testing, following the OECD 310 guideline, and changes in community structure were followed by Illumina amplicon sequencing in time. Removal of tested chemicals and their transformation products were determined by GC-FID and LC-MS/MS. Results of these experiments show differences in the biodegradation capacity between the tested inocula. Short term exposure reduced these differences and increased the biodegradation capacity for metformin and 4-chloroaniline. Long term exposure of activated sludge to n-methylpiperazine in chemostat cultures leads to an enhanced biodegradation of this compound. These results will allow a better understanding of the relationship between microbial adaptation and biodegradation performance. Moreover, microbial communities exposed to metformin were able to degrade its known persistent transformation product, guanylurea, which is persistent in fresh water. Ultimately they will also allow more realistic predictions of their biodegradation in the environment compared to those obtained using standard testing protocols.

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Microbial catabolism-based grouping enables read-across of the biodegradability of multiconstituents

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Ready biodegradability screening tests (OECD 301A-F and OECD 310) are used to assess the biodegradability of chemicals. In these test, a distinction between the biodegradability of an individual component of multi-constituent substances cannot be made because of the non-specific analysis used. REACH requires assessment of the biodegradability (persistency) of all constituents present at ≥ 0.1 wt% in a substance to determine their PBT/vPvB properties. Fatty acid amides (FAA) and alkylpolyglucosides (APG) are surfactants containing many constituents. Ready biodegradability for a number of these surfactants was demonstrated using the OECD screening tests. Testing the biodegradability of all constituents present in these surfactants is difficult or even impossible due to the large number of constituents and/or the unavailability of the individual constituents. Read-across is a non-testing method to predict biodegradability and considered to be a good alternative to experimental biodegradability data. The grouping of individual surfactant constituents for the read-across of biodegradation test endpoints is justified by using information on the catabolism of the microorganisms involved in the growth-linked biodegradation. FAA and APG degrading bacteria were isolated from inocula also used in ready biodegradability screening tests. The aerobic biodegradation pathways and the substrate specificities of the isolates capable of growing on these surfactants have been studied. It was shown that the (primary and secondary amide) FAAs and APGs were biodegraded starting with hydrolysis of the amide and glucosidic bond, respectively. The broad substrate specificity of the bacterial isolates involved in the first biodegradation steps and the subsequent rapid biodegradation of products formed allows read-across of ready biodegradability for all constituents present in the surfactants. In conclusion: Microbial catabolism-based grouping of constituents justifies read-across thereby enabling (improving) the ready biodegradability assessment of multiconstituent substances and UVCBs.

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Non-extractable residues, an overlooked new hazard in the persistence assessment?

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Non-extractable residues (NER) so called "bound residues" of plant protection products are formed *e.g.* in soil as a result of degradation processes. Due to their inherent nature, analyses and further assessments of bound residues are challenging. If the formation is due to microbial degradation processes the nature of the NERs is of course different to nature of original parent compound and release of NER is very slow in time and strongly correlated to degradation processes of the matrix (*e.g.* soil). In 2014 a new classification of non-extractable residues has been evolved and the non-extractable residues have been classified into three types of: NER type I: Entrapped, strongly adsorbed, no covalent binding NER type II: Covalently bound non-extractable residues NER type III: Biogenic bound residues ECHA published some guidance for the assessment of (Persistent Bio-accumulative and Toxic) PBT properties in 2017 (ECHA_2017_R.7b; ECHA_2017_R.7c; ECHA_2017_R.11). In addition in 2018 a discussion paper improving the interpretation of non-extractable residues (NER) in degradation assessment has been published by ECHA. I this discussion paper methods have been evaluated, how non extractable residues could be further experimentally analysed. In the regulatory view of ECHA the non-extractable residues are regarded as completely bioavailable and non-degraded residues in the regulation of general industrial chemicals and therefore they are regarded and handled in the risk assessment as parent substance if not shown otherwise experimentally. From our experiments it was concluded that the experimentally elucidation of non-extractable residues is highly sophisticated and therefore still challenging. However, even with soil destroying methods like the silylation, enzymatic digestion or digestion with 6 molar HCL at 110°C the main portion of bound residues will remain bound to the soil and therefore it could be concluded that this part is not bioavailable. The liberated non-extractable soil bound residues could not be assigned to bromoxynil nor any metabolites of bromoxynil. A small could be assigned to natural amino acids. Both results indicate that bound residues will not contribute to future risks or hazards because they are on one hand not bioavailable or on the other hand they have been converted to natural biogenic residues like natural amino acids. On the basis of these results an overlooked new hazard would not be foreseen.

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From Bioavailability Science to Regulatory Persistence Assessment

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Bioavailability science has advanced significantly over the past 30 years. A recent article published after a SETAC special science symposium concluded that the science was sufficient for bioavailability concepts to be incorporated into regulation in order to provide for better risk assessments, through incorporation of risk-based measures of bioavailability and bioaccessibility. Under REACH, persistence (P) – as part of PBT/vPvB – assessment is largely hazard-based and does not recognise bioavailability concepts. This is exemplified by recent developments around characterisation and interpretation of non-extractable residues (NER). However, bioavailability is important as a lack of bioavailability prevents microbial degradation from occurring. Bioavailability of substances can also vary greatly depending on factors such as means of application, soil characteristics, extent of aging and source material. This presents difficulties in interpretation of P data and can lead to huge variation in derived half-lives, particularly from non-standard methods. Furthermore, it is arguable that the P assessment should focus on the (potentially) bioavailabile fraction of the substance in order to understand its intrinsic biodegradation potential and the recalcitrance of the fraction that may pose a risk to ecological receptors. This presentation aims to explore the potential for bioavailability science to be incorporated into regulatory P assessments, with a focus on phenanthrene as an example case. Phenanthrene represents an extreme case in that a vP classification has been proposed based on half-lives from certain field studies in soil, whereas in other studies the substance is demonstrated to be extensively degradable, particularly in water, and has even passed a ready biodegradability test. Considerations of bioavailability have been applied to the available P data with the aim of distinguishing the influence of lack of bioavailability from intrinsic biotransformation potential on derived half-lives. Particular focus has been placed on data generated according to the ECHA ITS, and how this relates to other data. Hazards and intrinsic biotransformation potential of the substance are also considered in assessment of risks posed from remobilisation of residual and NER fractions. Finally, implications for interpretation of weight of evidence and remaining uncertainties are explored, and recommendations for future assessments are proposed.

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Poster spotlight

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Communicating Science, Failures, Fortune and Risks to Improve the Value of Environmental Research to Society (I)

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Data management system upgrade to ensure reasonable assurance level of non-financial reporting
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A constantly increasing number of investors and stakeholders are seeking for key information in the area of sustainability. This leads to an increasing demand for a transparent communication of non-financial data of companies. Sustainability reporting is a communication tool for stakeholders regarding a company's managerial performance and likelihood to continue business. External assurance of non-financial information is also increasingly relevant to ensure the quality and accuracy of information conveyed. In the case of Health, Safety and Environment (HSE) related information, to cope with increased disclosure expectations and to ensure high standard of data quality, a standardized data processing management system needs to be implemented. The goal of this paper is to evaluate the status quo of a company's HSE data processing system in order to identify challenges and opportunities to implement a data management system corresponding to a reasonable assurance level. The analysis considers environmental information related to energy input, water consumption, waste generation and Carbon dioxide emissions. The company under consideration in this analysis is a multinational performing integrated annual reporting and seeking reasonable assurance level for sustainability data. The outcome and methodology in this analysis can be implemented in other companies to improve and standardize their existing environmental management system in line with reasonable assurance level requirements. Although, the highest performing site scored 80% of available points indicating it already has satisfactory HSE data management implemented, close to 50% of sites investigated were found to have a weak data management system with urgent need for improvement. Results show that internal controls are implemented for over 90 % of all HSE aspects investigated, but only half of them meet the requirements and expectations of a reasonable assurance statement. There is therefore a great potential for improvement in the areas of data processing, documentation, internal communication and data validation at critical process points through the implementation of a reliable internal control system. In addition, the importance in adopting an IT system capable of standardizing the interfaces of data capture from external providers emerged as a critical aspect in upgrading the HSE data processing system. \n

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Transparency within REACH?
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According to REACH, the chemical industry must ensure that risks from chemicals they produce or import can be adequately controlled. Data on the chemicals' properties and uses, hazards and risks as well as instructions for safely handling, must be provided before chemicals are allowed on the market. The information is used to identify chemicals that warrant regulation. Thus, the registered data need to be reliable and relevant as well as transparently reported to ensure that chemicals of concern can be identified. In this presentation, results from four papers evaluating the transparency of REACH will be discussed. The papers report on lessons learned, and possible societal impact, from how the European Chemicals Agency and the chemical industry communicate scientific data for substances regulated under REACH. The results show that information that is used for concluding on hazards and risks of chemicals as well as industry's conclusions are reported in a semi-transparent manner and therefore difficult for third parties to fully scrutinise. This was in part due to the protection of confidential information but also related to ECHA's procedures for making information available as well as industry's reporting. Furthermore, industry is only required to summarise (eco)toxicity studies that are gathered for the risk assessment. Consequently, data based on industry studies that are not publicly available cannot be scrutinised by third parties. Thus, the system relies on studies being accurately summarised by the registrant although this was not always seen to be the case. Furthermore, the current framework for industry to evaluate (eco)toxicity studies and report data evaluations were found to be neither systematic nor transparent. Studies may not be evaluated based on their inherent scientific quality when the Klimisch method for evaluating data is used. Using the Klimisch method may also result in giving less weight to non-standard studies, such as many academic research studies, although non-standard studies could contribute with important information to the risk assessment. The transparency of data evaluations could be improved by using a framework that has clear criteria and guidance as well as a structured format for reporting data evaluations. This would support more harmonised and transparent data evaluations and encourage studies to be evaluated according to their inherent scientific quality rather than mere compliance with standardised guidelines.

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Breaking down the barriers to science - Providing access to industry safety studies
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Crop protection products are among the most thoroughly researched and strictly regulated chemicals but people are worrying about their use and what it means for human health and the environment. Companies have not typically shared their safety studies with the public, which has often been a point of debate. Although some authorities make study summaries available on their website, information remains largely inaccessible to the public. Bayer is committed to restoring trust in the integrity of crop protection science and has decided to open up our science for everyone. By doing so, Bayer aims to foster an open, transparent, and science-based dialog around crop protection products. The transparency website aims to reach both interested layman and the scientific community. Visitors can access safety-relevant summaries and evaluations of active substance (a.s.) studies used in Bayer products and request full study reports. Disclosure is a continuous process, starting with a.s. registered in the EU and other countries (covering >90% of studies on an a.s. globally). Documents are redacted to comply with data privacy rights of Bayer's employees or contractors and to protect information related to details prone to commercial misuse. All blackened out data of course have been disclosed to and assessed by regulators. \n As a second pillar, videos, infographics and education material is provided to put regulatory science into context and to help break down barriers to science. Launched in 2017, Bayer's transparency initiative was the first in the industry to proactively enable access to safety-relevant information on its crop protection studies. Since its start, documents for 28 actives and many representative products have been made available. So far, more than 5000 site visitors and 17 requests of full study reports have been received. Transparency is the cornerstone of our philosophy as a life-science company. We fully support efforts to improve transparency around crop protection safety studies and recognize our responsibility to communicate how we assess our products' safety. We are reaching out for scientific collaboration to advance research in the fields of agronomics and public health. At the same time we want to level the playing field by providing background material and explanations, empowering non-scientists to be part of the debate around modern crop protection.

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Ecopharmacovigilance of human medicinal products: Assessing and managing the real world risks resulting from patient use
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Ecopharmacovigilance (EPV) is a developing science concerned with the detection, evaluation, understanding and prevention of adverse effects of active pharmaceutical ingredients (APIs) in the environment. In Europe and North America, a new regulatory submission or a line extension is normally accompanied by an Environmental Risk Assessment (ERA), but there is no regulatory requirement to monitor the environmental risks of medicines post-launch. Nevertheless, researchers globally continue to report instances of pharmaceutical residues in the environment, and publish new ecotoxicological studies on effects of pharmaceuticals on wildlife. To understand the significance of these emerging data and ensure that any potential risks are identified and managed appropriately, we have developed a process for EPV. This ensures that we can continue to understand the environmental risks associated with our products, and to manage them appropriately throughout the life of each product. This presentation will describe our EPV processes, how it works in practice and present a semi-probabilistic and spatially explicit environmental risk assessment for the patient use of several cardio-vascular and oncology APIs detected in the environmental surface waters. EPV is the environmental equivalent of pharmacovigilance and allows the environmental impact of ongoing patient use to

be managed though (i) routine literature monitoring for emerging data, (ii) the update of an ERA where emerging data challenge the original assumptions, and (iii) highlighting when APIs are detected in the environment at levels above published PNEC. EPV to date indicates that these instances represent < 2% of the total reported concentrations indicating site specific rather than generic risks that can be attributed to low flow, low dilution or poor wastewater treatment.

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Improving transparency, consistency and efficiency of ecotoxicological teaching: launching the newly developed Open Online Textbook Environmental Toxicology

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Although several textbooks are available, teaching environmental toxicology in general seems to suffer from a lack of a well-elaborated, up-to-date and consistent textbook that covers all aspects of the field. As a consequence, every university is developing its own training materials in addition to a textbook, but only little of this material is available online. And materials online often are not consistent, lack novelty or do not cover the entire field. We took the initiative to develop an open online textbook on Environmental Toxicology that should cover the field in its full width, including aspects of environmental chemistry, ecotoxicology, toxicology and risk assessment. The initiative is sponsored by the Netherlands Ministry of Education. We aim at developing an online open access book on Environmental Toxicology that is useful for training at BSc, MSc and higher levels. The book is designed in a modular way, each module having clear training goals and flagged with a number of keywords. The book will also contain tools for self-study and training, like questions and exercises. With the publication of the open online book, we aim at improving quality, continuity and transparency of the education in environmental toxicology. We also want to make sure that fundamental insights on fate and effects of chemicals gained in the past are combined with recent approaches of effect assessment and molecular analysis of mechanisms causing toxicity. To guarantee quality of the book and associated training materials, each module is authored by 1-2 experts and reviewed by at least one international reviewer from outside the project team. An advisory board is involved in supervising the project, as well as educational advisors, while the project team serves as an editorial board. The project team, consisting of environmental toxicologists and chemists from six Dutch universities, also has involved colleagues from within the SETAC community to write modules that required specific expertise not covered. With that, we hope we can produce a book that is written and supported by SETAC, that is covering the entire field, and is useful for training within e.g. the SETAC Europe Certified Risk Assessor (CRA) programme. The publication as an open online book will allow continuous updating of the book, providing a possible role of SETAC in sustaining the book. At the SETAC Europe Annual Meeting in Helsinki we will launch our open online textbook on Environmental Toxicology.

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A Scientific Perspective on Microplastics in Nature and Society

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Nano and microplastics (NMPs) are plastic particles of mixed shape that are present in air, soil, freshwater, seas, in biota, and in several components of our diet. NMPs come from a variety of sources, including products and textiles (use and breakdown), fisheries, agriculture, industry, waste, and others. Scientists, policy-makers and the public are becoming increasingly concerned about both the ubiquity of NMPs and the uncertainties surrounding their impacts, hazards and risks to our environment and to human health. Traditionally, the topic of NMPs has been addressed within separate scientific disciplines, but the consensus is increasingly that we need interdisciplinary approaches to understand the impacts and implications of NMP for the environment and society and to understand how to use this complex evidence base to help define policy and find solutions. Here we report on the process and outcome of a group of scientists writing a rapid Evidence Review Report for SAPEA (Science Advice for Policy by European Academies), as part of the European Commission's Science Advice Mechanism. The report, to be released in January 2019, offers a scientific perspective on the state-of-the-art knowledge about the implications of NMPs in nature and society and highlights the features and complexities of the topic. A unique aspect of this report is that it reviews relevant evidence from the social and behavioural sciences (e.g., on behaviour change, risk perception, media coverage), in conjunction with the current natural sciences evidence (e.g., on sources, occurrence, hazards, risks), which is crucial to designing effective policies. It also highlights uncertainties and knowledge gaps in order to inform appropriate future actions. Evidence from the environmental and health sciences, computer modelling, social, behavioural and political sciences are reviewed and presented together from a multidisciplinary perspective. The SAPEA Evidence Review Report informs a forthcoming Scientific Opinion by the European Commission's Group of Chief Scientific Advisors, which will contain policy recommendations and will be released in June 2019.

Scientific Advancements towards Risk Assessments, their Frameworks and the Implementation of Alternative Strategies to Animal Testing for Nanomaterials

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Chronic effects of Safer by Design CuO NMs on young adults of the snail, Lymnaea stagnalis: influence of coatings and dispersants

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Copper oxide nanomaterials (CuO NMs) are frequently employed for their antimicrobial properties in antifouling paints and other applications. Their extensive use can lead to contamination of aquatic ecosystems, where they can undergo further physico-chemical transformations. The main objective of this study was to investigate the chronic sublethal toxicity of different CuO NMs to the great pond snail *Lymnaea stagnalis*, a representative organism of the benthic ecosystem. It has become increasingly recognised that sublethal effects as measures of toxicity, are far more sensitive in the assessment of the potential impact of chemicals within an ecosystem. Chronic sublethal concentrations were selected based on the data gathered from acute exposure studies. Experiments aimed to investigate the effects on the reproduction and growth of *L. stagnalis* following exposure to Cu, either as ionic Cu ($CuSO_4$), pristine CuO NMs or "safer by design" CuO NMs coated with ponivinilpirrolidone (PVP), functionalised in either Milli-Q water or phosphate buffer (PBS). Results indicate a strong influence of the different dispersant used in the toxicity of CuO NMs. Indeed, when comparing the chronic lethal toxicity of all the CuO NMs tested at sublethal concentrations (0-200 μgL^{-1} Cu), findings showed an increase in toxicity due to the presence of PBS, which promoted further the agglomeration of the NMs. At that concentration range, no toxicity was recorded after exposure to pristine CuO NMs (H_2O). In contrast, CuO-PVP(PO_4^{3-}) NMs and CuO(PO_4^{3-}) NMs exposures exhibited a higher mortality after 30 days, with an LC_{30} estimated as 186 μgL^{-1} Cu for exposure to CuO(PO_4^{3-}) NMs and LC_{50} of 160 μgL^{-1} Cu for experiments with CuO-PVP(PO_4^{3-}). Findings from the sublethal endpoints indicated a direct link between the feeding rate inhibition and the decreased fecundity. This was confirmed by results gathered from the exposure to CuO-PVP(H_2O) NMs, where snails exhibited no alteration in feeding rate in response to increasing concentrations of the NMs and thus subsequently reproduction and growth were not affected in a concentration-response manner. This suggests that PVP, in absence of PBS, was able to mitigate the toxicity of the core CuO NMs in the long-term exposure.

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Nanomaterial characterization - which parameters are needed from a regulatory point of view and how do we measure them?

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In risk assessment, physicochemical characterization of the nanomaterial is an essential first step. This is particularly relevant where grouping, read-across and categorization of nanomaterials is used, i.e. where identification of similarities and differences is key. Within OECD a number of physico-chemical parameters have been identified as relevant to and important in the assessment of nanomaterials. The *Physico-Chemical Decision Framework to Inform Decisions for Risk Assessment* is the next step in the development of guidance for physico-chemical

characterisation and, where necessary, the development of new test guidelines. Together with the *Guiding Principles for Measurements and Reporting for nanomaterials: Physico-Chemical Parameters* it will assist users in deciding on physicochemical parameter(s) relevant to their nanomaterial and purpose, as well as identifying the most suitable technical method(s) and their reporting requirements to determine these parameters for their specific nanomaterial and purpose. Based on the framework and guiding principles, the presentation will discuss which physicochemical properties are relevant for characterization of nanomaterials from a regulatory point of view, address suitable – and less suitable – methods to test those properties, and identify future needs for standardization and harmonization.

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The nanoGRAVUR framework for grouping of (nano)materials based on a harmonized set of material properties with 34 case studies

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Nanomaterials are commercialized in a myriad of grades that are optimised in composition, size, shape, coating for specific applications. As the scope of the different reporting and registration schemes in EU countries, in the USA and Canada includes nanoforms of widespread materials, the need arises to evaluate if the different grades can be grouped for risk assessment purposes. Additionally, there is necessity to ensure the safe use of innovative nanomaterials early during R&D, ideally by read-across without animal testing. The project nanoGRAVUR (BMBF, 2015-2018) developed a framework for grouping of nanomaterials. Different groups may result for each of the three distinct perspectives of Occupational, Consumer and Environmental safety. Each is assessed by a risk matrix that integrates hazard and exposure indicators. The indicators are harmonised between the three perspectives and are based: · tier 1 on intrinsic physical-chemical properties (what they are) or GHS classification of the non-nano form (human tox, ecotox, physical hazards); · tier 2 on extrinsic physical-chemical properties, release from nano-enabled products, in-vitro assays with cells (where they go; what they do); · tier 3 on case-specific testing, potentially in-vivo studies to substantiate the similarity within groups or application-specific exposure testing The methods developed by nanoGRAVUR fill several gaps highlighted in the Steinhäuser & Sayre (2017) reviews, and are useful to implement both the ECHA concept of nanoforms as well as the EPA concept of discrete forms. Case studies include families of Fe_2O_3, SiO_2, CeO_2, organic pigment, ZnO, Cu, TiO_2 (nano)forms. Nano-enabled products included cardboard, automotive coatings, cements, plastics. Benchmark nanomaterials and benchmark nano-enabled products are essential to achieve reproducible groupings across different labs with slightly differing equipment (e.g. for dustiness, sanding, dispersion stability, reactivity). Furthermore, benchmark materials span the dynamic range of each property (often about three to five orders of magnitude), and thus help to assess the relevance of any dissimilarity between different nanoforms.

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The GRACIOUS draft Framework for the Grouping of Nanomaterials in order to streamline risk assessment and decision making

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Nanomaterials exist in a variety of nanoforms characterised by differences in properties such as size, shape or coating. In order to reduce the need to assess risk on a case-by-case basis, the GRACIOUS project will develop a framework that groups nanoforms together. The initial steps require basic existing or predicted information relating to the purpose of grouping (e.g. regulatory risk assessment or product innovation), the physicochemical properties, the intended uses and the potential releases of the nanoform(s). The GRACIOUS framework then provides a structure that uses this basic information to formulate hypotheses that explain why certain nanoforms can be grouped in relation to a specific hazard endpoint. These hypotheses consider information about; · what the nanoforms are (physicochemical characteristics), · where they go (in the environment, within the body), · what they do (hazard), and · the intended use (life cycle stages that result in release and exposure). The framework allows identification of whether the nanoform(s) align with a range of evidence based **well defined hypotheses**, for which the risk implications are clear. If the nanoform does not fit, then the framework guides the user through generation of a **user defined hypothesis** for grouping. The justification whether a grouping hypotheses applies to a specific (group of) (nano)form(s) is investigated via a tailored Integrated Approach to Testing and Assessment (IATA). The IATA guides the user through identification of relevant existing information, *in silico* and testing needs. In this manner, the GRACIOUS framework facilitates scientifically sound risk decision making for nanoforms, while minimising case-by-case testing. *Acknowledgement* – GRACIOUS is funded by the European Commission, Grant Agreement 760840.

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Towards validating nanomaterial predicted environmental concentrations from SimpleBox4nano using the NanoFASE spatiotemporal multimedia fate model

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For prospective environmental risk assessment the predicted environemntal concentration (PEC) is not based on measurements but on calculations using modelling tools for estimating a substances release and fate in the environment. One of such tools is the screening level multimedia fate model SimpleBox. This model has recently been adapted for use with nanomaterials (SimpleBox4.0-nano (SB4N)), because nanomaterials require the inclusion of some physico-chemical processes such as agglomeration and dissolution to describe their fate in the environment. Although SB4N has undergone thorough testing, i.e. of its sensitivity to the defining nanospecific parameters, it remains unknown to what degree the steady state approach to modelling background concentrations compares to the expected temporal and spatial variability of nanomaterial PECs. With the recent development of the spatiotemporal multimedia fate model within the NanoFASE project such a comparison is now possible. The study of these two modelling approaches has two goals, first to indicate if the screening level PEC is a valid PEC at the regional scale and second if any further adaptions are required in order to improve the predictability of the model for nanomaterials. The SB4N and NanoFASE (NF) multimedia fate models where applied to the same spatial scenario: the Thames catchment. SB4N using minimal spatial resolution for a screening level exposure assessment and the NF model with much more spatiotemporal detail (5x5km grid). Overall the results show the expected outcome of steady state concentrations using a big mixed box. On the one hand for the water compartment the singular mixed box approach slightly underestimates the PEC in water. This can be mended by dividing the water compartment into two based on the distribution of nanomaterial emission to up and downstream streams. On the other hand, because of the PECs being at steady state, a compartment that acts as a sink, will continue to accumulate a lot of nanomaterials. This is the reason why the PEC from SB4N in soil is so much higher than the soil PEC from the NF model after one year.

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Surface hydrophobicity of nanomaterials as surrogate of the Kow approach: a test method by JRC based on the binding affinity to engineered collectors

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Information on physicochemical properties influencing the fate and behaviour of chemicals in the environment or their toxicokinetics in the human body is crucial for chemical risk assessments in any regulatory context and jurisdiction. Several regulations, including REACH, require reporting the octanol/water partitioning coefficient (Kow), an indicator of the fate and transport of a chemical in the aquatic compartment and a key parameter of any environmental exposure model estimating sorption to soil and sediment, bioavailability and bioconcentration/bioaccumulation in aquatic organisms. Kow also plays a role in (eco)toxicity predictions, e.g. in (Q)SAR modelling. It has been explained in the scientific literature that (insoluble) nanoparticles, due to their colloidal behaviour, cannot reach the thermodynamic equilibrium when partitioning between the octanol and water phases. For this reason, the use of Kow in nanomaterial risk assessment may lead to erroneous conclusions and scientists are investigating more appropriate fate predictors for nanospecific environmental exposure models. The OECD Working Party on Manufactured Nanomaterials (WPMN) concluded that the existing Test Guidelines for Kow determination are not applicable to (insoluble) nanoparticles and pointed out that a surrogate of the Kow may be necessary for obtaining information on nanomaterial environmental behaviour. This presentation illustrates a new test method for quantifying the polar component of the surface free energy of nanoparticles by measuring their binding affinity to rationally engineered hydrophobic and hydrophilic surfaces (collectors), via analysis of their adsorption kinetics by Dark-Field microscopy and using the XDLVO (eXtended Derjaguin Landau Verwey Overbeek) theory. The polar component of the surface free energy is a direct measurement of the surface hydrophobicity of solids. Determining the surface hydrophobicity of nanomaterials presents a reasonable alternative to the Kow for fulfilling regulatory information requirements, evaluating bioaccumulation potential (e.g. for PBT assessment) and predicting environmental fate and concentrations in exposure models, thus also providing a means to reduce animal testing.

Linking Models, Experiments and Measurements to Reliably Investigate the Environmental Fate and Effects of Hydrophobic Organic Contaminants and Mixtures (I)

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Vertical profiles of hydrophobic organic contaminants (HOCs) in the sediment-water interface - assessing the impact of organic carbon cycling on HOC fate in the marine environment

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Environmental fate of hydrophobic organic contaminants (HOCs) is closely linked to cycling of organic carbon. Organic carbon rich sediments often act as a sink for HOCs, but as primary emissions of legacy HOCs decrease, sediments may start acting as a secondary source to the water column. In this project, we hypothesize that organic carbon origin (i.e. terrestrial versus marine) and thus its sorption capacity, affects sediment-water fluxes of HOCs. To study the impact of organic carbon cycling on sediment-water fluxes of HOCs, four sites with in the Baltic Sea and a Norwegian Fjord, with different primary production regimes and relative contributions of terrestrial versus marine organic carbon, were sampled. Equilibrium passive samplers mounted on a recently developed sediment-water interface probe were used to sample freely dissolved HOCs. The organic carbon origin at the different sites was determined using stable carbon isotope signatures ($\delta^{13}C$). Data from the Norwegian site (representing low input of terrestrial organic matter) exhibited a high resolution profile of the sediment porewater ($C_{free\text{-}porewater}$)-bottom water ($C_{free\text{-}bottom\ water}$) interface for a wide range of HOCs (PCBs, PAHs, pesticides and chlorobenzenes). A strong concentration gradient between sediment pore water and overlying bottom water was observed for several PAHs indicating a potential flux of the chemicals from sediment to water. Further data will provide insights into effects of the different organic carbon cycling on HOC fate at the investigated sites.

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Measurement of Freely Dissolved Concentrations of Alkylated PAHs in Sediment Porewater Using Solid Phase Microextraction with PDMS Fibers

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Background: Application of the U.S. EPA equilibrium sediment benchmark approach for PAHs typically relies on total concentration (C_{total}) measurements of 18 parent and 16 alkyl polycyclic aromatic hydrocarbons (so-called 34 PAHs) in sediments. Following organic carbon normalization, these data are used for calculating the sum of PAH toxic units (∑TU) for assessing risks to benthic organisms. However, equilibrium partitioning assumptions that are inherent in this procedure may overstate potential risks which can be more reliably assessed using freely dissolved concentrations (C_{free}) in sediment pore water. Recent advances in equilibrium passive sampling methods (EPSMs) offer a promising alternative to C_{total} since bioavailability of sediment PAHs can be directly quantified via C_{free}. Currently, there is a growing consensus that C_{free} measurements provide an improved technical basis for sediment quality and risk assessment. However, to apply EPSMs reliable polymer to water partition coefficients are crucial. **Approach:** A laboratory (*ex-situ*) equilibrium passive sampling method was developed for evaluating the risk of parent and alkyl PAHs in sediments. The method relies on solid phase microextraction (SPME) with different silicone materials, i.e. polydimethylsiloxane (PDMS) coated glass fibers and hollow fibers. To date polymer-water partitioning coefficients are only available for parent PAHs for different polymer materials including PDMS. The first study objective was to determine PDMS-water partition coefficients (K_{PDMS}) for a range of alkylated PAHs. Based on the experimental values obtained, a Log K_{PDMS}-Log K_{ow} regression was developed and applied to estimate K_{PDMS} for a wide range of alkylated PAHs. The second objective was to apply this method on surficial sediments collected at seven sites in the Baltic Sea off the coast of Germany. The third goal was to compare C_{free} data from the *ex-situ* method to corresponding PAH measurements obtained at these same sites using *in-situ* deployment of PDMS passive samplers. **Results:** Reliable K_{PDMS} partition coefficients for alkylated PAHs were generated using 3 different PDMS coated fibers thereby extending use of EPSMs for this important class of contaminants. C_{free} obtained using *ex-situ* and *in-situ* approaches were comparable with mean values typically within a factor of 3. These results support further use of ex-situ based EPSMs which are quicker and likely cheaper than field deployments.

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Biodegradation testing of volatile chemicals in water-sediment systems (OECD 308)

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OECD 308 guideline is used for biodegradation testing of chemicals in water-sediment systems. The guideline mentiones that it is not suitable for testing volatile chemicals and recommends a closed flask setup for testing slightly volatile chemicals. However, a clear guidance on system geometry and construction of the recommended test setup is lacking. In such tests, the choice of system geometry and sediment:water (S:W) ratio influences the partitioning and hence degradation of test chemicals. Additionally, it needs to be addressed how volatilisation should be considered in such tests during data treatment. Our objective was to adapt recently developed improved test setups to conduct OECD 308 test using four ^{14}C labelled volatile chemicals with different volatilisation range (phenanthrene < biphenyl < tetralin< decane). The data obtained were used to understand the underlying processes and suggest possible data treatment and data reliability measures.Two types of sediment were used. The test setup comprised of 50g (dry weight) sediment in a 500 mL vessel (Cylindrical, Ø= 5 cm) with location water with a S:W ratio of 1:3 and 1:4 for the high OC (Organic Carbon) and low OC sediment sample, respectively. The setup consisted of a permanently connected tenax tube and an internal NaOH trap to capture volatilized and mineralized fractions. The samples were applied with test chemicals using an appropriate co-solvent. Every week the headspace of samples was oxygenated to 18-20% oxygen saturation. At sampling the sample bottle was opened only after headspace stripping of air through the tenax tube. The tenax tube and NaOH trap were taken for analysis. The overlaying water was separated using a pipette and both sediment and water phase were further taken for extraction and further analysis. The sediment phase after extraction was allowed to dry and was combusted to determine the non-extractable residues (NER). The average mass balance was 91.3% ± 7.3 (S.D. N= 48), 99.4% ± 7.0 (N=46), 86.4 % ± 6.93 (N=50) and 93.2% ±9.9 (N=44) for phenanthrene, biphenyl , tetralin and decane studies. Generally, we observed that system geometry, S:W ratio used, had an influence on the partitioning of the test chemicals. Formation of biofilms on the overlaying water phase was very characteristic to closed setup applied with co-solvent (except sterile samples). We suggest reporting of procedural recovery, which improves the data reliability in the context of testing volatile chemicals.

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Passive equilibrium sampling in aquatic biota: a glimpse on the bioaccumulation of hydrophobic organic compounds and its relation with trophic level

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Passive Sampling Devices based on polymers such as silicone, if operated in equilibrium mode, offer a superior approach to assess bioaccumulation than traditional exhaustive extraction, since they avoid the need for normalization of the total concentrations of hydrophobic organic compounds to the main sorptive phase, e.g. lipids. Recently, a new approach for equilibrium passive sampling in lean tissue has been proposed[1,2], allowing to reach equilibrium in biota tissue with low to medium lipid content, and thus to compare a set of species across trophic levels. In this study, a well characterized closed ecosystem has been selected, a small Swedish lake (Ången), and five different biota species were studied: crayfish, perch, roach, bream and eel. For crayfish and eel, the selected tissue was the muscle. For perch, roach and bream, the entire fish were analyzed. The samples were homogenized and the lipid content was determined. In order to calculate the trophic level, $\delta^{15}N$ was also determined. The homogenized tissues were sampled using silicone with three different thicknesses: 125, 250 and 350 μm to confirm equilibrium partitioning between the samples and the silicone, relocating the samplers 6 times per day over 5 days. The silicone was extracted, and the extracts were cleaned-up using EMR-Lipid cartridges (Agilent Technologies). The analysis and quantification of a wide range of compounds, including PAHs, PCBs, PBDEs, pesticides, musk compounds, sunscreens and antioxidants, were achieved with a gas chromatograph coupled to a high-resolution mass spectrometer (GC-HRMS Orbitrap Q-Exactive, Thermo Fisher). The present work accomplished, for the first time, the study of 160 compounds using passive equilibrium sampling in biota with different lipid content, from lean tissue to lipid-rich tissue. The equilibrium was confirmed for the compounds quantified in the different tissues, using silicone of different thicknesses. Despite the comparison of different tissues (muscle and entire body) from different species and the use of a standard $\delta^{15}N_{base}$ for calculations which might blur the interpretation of results, for some compounds we observed trends of increasing chemical concentrations with increasing trophic level. The aforementioned uncertanties will be reduced during the further implementation of the project, studying a complete set of samples from this ecosystem. [1] Rusina et al, 2017.

Environ. Sci. Technol. 51(19),11250-11257 [2] Rojo-Nieto et al. *Under review*

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Monitoring of POP in biota versus aqueous passive sampling - a comparison in equal units

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Persistent organic chemicals pollutants (POP) such as PCB, hexachlorobenzene, organochlorine pesticides, PBDE, etc, are still present in surface waters and monitoring a number of them is required under the EU Water Framework Directive. Aquatic biota was selected as matrix for compliance monitoring of POP. However, concentrations of POP in fishes depend on size, age, sex, lipid content and physiological condition, as well as the season. For small water bodies around Europe, it is often not feasible to sample the same species, not even within a single catchment. The harmonization of biota monitoring thus presents a great challenge, and the WFD provided extensive guidance including correction for variations in dry-weight, lipid content, and trophic level (TL) of the monitored species. POP's concentrations in biota obtained for TL=4 by extra- or inter-polation using their log-linear dependence with TL, should be provided for checking compliance with environmental quality standard in biota (EQS_{biota}). The enrichment of stable isotopes ($\delta^{13}C$, $\delta^{15}N$) throughout the food web is commonly used to determine the trophic levels of species within a food web, while also generic values are applied, e.g. as listed at www.fishbase.org. Since POP accumulate in passive samplers their usage has been proposed as an alternative to chemical monitoring in biota. Passive sampling is frequently applied to estimate POP'sfreely dissolved concentration (C_{free}) in surface water and sediment pore water. Alternatively to C_{free}, uptake by passive sampling from various media, i.e. water, sediment and also fish tissue, can also be converted to a lipid based concentration ($C_{lip?media}$). These $C_{lip?media}$ are comparable in the same units with lipid based concentrations in fish tissue (C_{lip}) determined by conventional extraction. Such a comparison shows the differences in POP's thermodynamic potential between fish of various trophic levels and water. It also allows to assess if $C_{lip?water}$ determined by PS provides anequal level of protection compared to C_{lip}, obtained following the WFD guidelines. This work provides a basis for an informed decision on whether or not application of PS can comply with POP monitoring under the EU WFD. *Acknowledgement* - This work was supported by the Czech Science Foundation Grant No. GACR 15-16512S „Investigation of accumulation of persistent bioaccumulative toxic organic substances into aquatic organisms". \n\n

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Analysis of attenuating factors for terrestrial food web biomagnification of cyclic volatile methylsiloxanes

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Annex XIII of REACH indicates that a weight-of-evidence (WoE) determination on bioaccumulation (B) should consider "all available information" regarding this important environmental metric. Advances in the evaluation of a substance's ability to bioaccumulate or biomagnify in food webs have been published and are widely accepted by experts in this field, yet are not being consistently applied by regulators in PBT determinations. As indicated in the Stockholm convention, the critical concern regarding bioaccumulation is the issue of biomagnification, specifically substances that accumulate via the food web to progressively higher concentrations that may toxicologically impact top predators and humans. The key question is how to best predict biomagnification, a complex ecosystem phenomenon that is difficult to model in the laboratory. A laboratory BCF from water uptake is often used as a surrogate for biomagnification. But a BCF should only be considered a Tier 1, screening-level surrogate for biomagnification as it addresses only one level in the food chain and can be highly problematic in accurately characterizing dietary accumulation via the food chain for some substances. In this work, we examined the biomagnification of two volatile cyclic siloxanes, octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), in aquatic food webs that terminated with terrestrial organisms and compared data to a recent terrestrial food web study. Field and laboratory data on D4 and D5 indicate that despite having positive biota/water ratios, these materials do not generally demonstrate biomagnification (TMF>1) through analyzed aquatic/terrestrial food chains due to the ability for aquatic organisms to metabolize D4 and D5 and for air breathing organisms to clear D4 and D5 through respiratory exhalation and metabolism.

Towards a Science-based Risk Assessment Framework for Nano- and Microplastic

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Spatially resolved environmental emissions of seven commodity plastics in Switzerland

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While the knowledge on microplastic (MP) and macroplastic emissions is improving, uncertainties remain as to the local variations of MP concentrations. Large disparities are to be expected between residential, natural and agricultural areas among others, since specific uses and the corresponding emissions are dominating these environments. Identifying regions and compartments with potentially higher than average MP concentrations is crucial information to assess the possible risk in different settings. Moreover, the identification of possible hotspots may enable policy-makers to concentrate their efforts in more polluted areas. Using emission data available for Switzerland for seven different polymers, a regionalization of these emissions to a local scale is undertaken. The seven different polymers are: low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET). The resulting emissions are all expressed as probability distributions considering the confidence in the results. These emissions are then regionalized using different datasets as proxies for the considered emissions. The location of wastewater treatment plants (WWTP) and their level of treatment are used to model the release of MP and macroplastics from WWTP effluent and combined server overflow discharges. Maps of the population are used for the emissions of MP to outdoor and indoor air caused by private consumers and for the emissions of MP and macroplastics to residential soil caused by private consumers. Maps of the land use are used for the regionalization of further flows. High resolution maps of the quantiles of the emission distributions are then generated, giving best and worst case scenario estimates of the emissions of the different polymers to the environment. Different mapped datasets are considered as proxies to spread the modelled emissions over Switzerland, depending on the character of the emission. This regionalization will enable providing maps of MP and macroplastic emissions in Switzerland at high spatial resolution. These maps will show where the emission occurs and can act as first proxy to the plastic burden in Switzerland. Implementing fate processes based on these gridded datasets may then provide a more accurate estimate of the plastic pollution in Switzerland.

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Microplastics in terrestrial systems: Evidence for new potential effects

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Microplastics (plastics < 5mm, including nanoplastics < 0.1nm) are found globally due to fragmentation of large litter or direct environmental emission. Most of the research on this topic focused on impacts on aquatic systems, where several effects were reported. However, most of plastics arriving in the waters were produced, used, and disposed on land. It is within terrestrial systems that microplastics first interact with biota eliciting ecologically relevant impacts. We report here a suite of approaches including (I) a common garden experiment on the soil biophysical environment, (II) a greenhouse exposure of spring onions (*Allium fistulosum*) to 6 microplastics types, and (III) a climate chamber exposure of lettuce (*Lactuca sativa*) to 6 nanoplastic types. Such a combination of terrestrial models and microplastic particles allowed empirical evidence for new insights into the potential impacts of microplastics on unique properties of terrestrial systems. Microplastics altered the soil biophysical environment by changing the soil bulk density, water holding capacity, and the functional relationship between the microbial activity and water stable aggregates. Microplastics also triggered responses in spring onions' growth and traits, with significant changes in total biomass, above/below biomass ratio, root traits, and soil microbial activities. Effects of the tested polyester fibers and polyamide beads on plant traits and their function were the most pronounced. All the nanoplastic types significantly affected lettuce physiology (i.e. growth and biochemistry), with the magnitude depending on particle properties and exposure concentration (NH_2 termination and 50nm triggered the strongest effects). On this model, biochemical biomarkers suggested plastic uptake or close interaction of microplastics and lettuce roots. Confocal microscopy confirmed that most of the root fluorescence was due to nanoplastics strongly interacting with the roots surfaces. Our unprecedented results highlight the potential of microplastics to alter fundamental biophysical properties of the soil environment, which seem to be associated with responses on traits of spring onion. The smallest particles tested (nanoplastic) were also capable of causing significant plant toxicity. If extended to other models and plastic types, the processes unraveled here suggest that microplastics are relevant long-term anthropogenic stressors and drivers of global change in terrestrial ecosystems.

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Fate and impact of nanoplastic particles and microplastic fibers on a stream biofilm-grazer system

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Following the increasing global production of plastic, particulate plastics are increasingly detected in aquatic ecosystems, including fresh waters. Scientific information on their effects is therefore needed to evaluate the ecological and ecotoxicological significance of their occurrence in the water bodies. A better understanding of the trophic transfer potential of particulate plastics and its consequences are essential. Fate studies have identified the benthic compartment in the aquatic ecosystem as a target for the accumulation of particulate substances. In this study, we investigated the trophic transfer of nanoplastic particles and microplastic fibers (doped with a metal tracer which can be used as a proxy to more quickly and qualitatively evaluate plastic presence), from stream biofilms, a benthic microbial community, to a grazer upon dietary exposure. Biofilms were loaded with the plastic particles and fibers, and aquatic snails (*Physa acuta*) were allowed to graze on the contaminated biofilms for 14 days to assess the potential of plastic assimilation and consequent effects. Results showed that upon 24 hours of exposure, 60 % of the plastics added accumulated in the biofilms. This led to the alteration of the microbial composition and nutritive quality of the biofilms but did not impact their physiology or growth. The accumulated plastics in the biofilms were also transferred to the snails through their diet. However, no significant differences in the feeding rate of the snails was observed. On the other hand, growth rate of the snails feeding on the plastic particles and fibers was impaired and decreased by 30 ± 9 % in comparison to the controls. The reproductive output of snails, measured as the number of egg clutches, was also negatively affected when exposed to either nanoplastic or microplastic fibers. Indeed, snails feeding on control biofilms started to lay egg clutches on the fifth day of the experiment, while snails feeding on the contaminated biofilms did not reproduce at all. Due to ending the experiment, it was not clear if reproduction was only delayed or impossble for the exposed organisms. Overall, the present study confirmed an efficient transfer of particulate plastics and their negative impacts in an aquatic trophic chain, which can have consequences for trophic relationships and survival of invertebrates in aquatic ecosystems.

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Toxicity of nanoplastics to marine organisms
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The presence of small plastic particles in the environment is currently recognized as a global issue of concern. Nonetheless, the amount of small plastic particles in the aquatic environment continues to increase, in part due to ongoing increases in the production of plastics. A large number of organisms are exposed to these particles and this exposure may cause a variety of effects and threaten individuals of different species, the ecosystems they live in and, ultimately, humans [1]. An increasing number of studies have been providing information on the effects of microplastics. However, limited information has been so far provided in terms of nanoplastics, particularly to marine organisms. This work aimed to provide data in terms of effects to marine producers and a primary consumer (rotifers) of 50 nm polymethylmethacrylate nanoplastics, a polymer that has not been a subject of research. Additionally, a literature analysis of the available studies in terms of nanoplastics to marine organisms was integrated to derive a 5th percentile value (HC5) of nanoplastics of the most studies polymer. The effects of nanoplastics on microalgae were assessed throughout a 96h exposure in static conditions, testing a concentration range up to 304 mg/L. The effects of nanoplastics (4.7, 9.4, 18.9, 37.5, 75.0 mg/L) on rotifers survival was assessed in 48-h survival assays. Median effect/lethal concentrations were determined for producers and consumer, respectively. Overall, primary consumers were more sensitive to nanoplastics.

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Mimicking nanoplastic pollutants: study of their effects to biological systems
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The presence of micro- and nanoplastics in the marine environment is raising strong concern since they can possibly have a negative impact on human health. The lack of appropriated methodologies to collect the nanoplastics from water systems imposes the use of engineered model nanoparticles to explore their interactions with biological systems, with results not easily correlated with the real case conditions. Herein, we present a strategy for the fabrication of pure nanoplastics, the investigation of their physicochemical interactions with other aquatic pollutants and the toxicity evaluation of the specific products. In particular, we present a top-down approach, which, starting from bulk scale materials, allows obtaining nanosized particles, mimicking a degradation pathway more similar to the one occurring under real environmental conditions. The process, based on the laser ablation of polymer films in water, allows fabricating nanoplastics without the use of chemicals and precursors. The study is focused on polyethylene terephthalate (PET) nanoplastics, a material largely diffused and mainly used in the food packaging industry. We prove that the chemical and physical properties of the as-produced nanoplastics present similarities to the ones expected to be present in the environment. After the evaluation of the stability in various biological media, detailed studies on the nanoplastics interaction with three representative acquatic pollutants (mercury metal ions, levofloxacin antibiotic and glyfosate pesticide) have been performed. We prove that the nanoplastics interact succesfully with all three pollutants. The study of their interactions with Caco-2 and THP-1 cell lines reveals the presence of the nanoplastics is not toxic for the cells in the short term. However, the metabolomics study performed reveals that there is a significant effect of the presence of the nanoplastics and of the nanoplastic-pollutants in the metabolism of the cells, proving the adverse effects that these nanopollutants can cause to the biological systems.

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Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin
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Humans are potentially exposed to microplastics through food, drink, and air. While the first two pathways have received quite some scientific attention, little is known about the latter. A crucial aspect of the presence of atmospheric MP is related to its likelihood of being inhaled and potentially reaching the alveoli of the lungs. To date, only a few studies have addressed the potential human exposure to airborne microplastics. The present study extends knowledge of indoor airborne MP exposure, composition, and size ranges, providing data on particles (including fibres) of sizes down to 10 μm. We addressed the exposure of humans to indoor airborne microplastics using a Breathing Thermal Manikin (BTM). Three apartments located in Aarhus (Denmark) were investigated collecting a total of mine samples, then analysed using FPA-μFTIR-Imaging spectroscopy followed by automatic analyses (MPhunter) down to 10 μm particle size. All samples were contaminated with microplastics, with concentrations between 1.7 and 16.2 particles m^{-3}. Synthetic fragments and fibres accounted, on average, for 4% of the total identified particles, while nonsynthetic particles of protein and cellulose constituted 92% and 4%, respectively. Polyester was the predominant synthetic polymer in all samples (81%), followed by polyethylene (5%), and nylon (3%). Fragments resulted more abundant than fibres in the samples (87% and 13% respectively). Microplastics were typically of smaller size than nonsynthetic particles, and a correlation between MP size and their abundance was recorded ($R^2 = 0.702$), highlighting that the smaller the size, the higher the concentration in the air. As the smallest identified microplastics can be inhaled and possibly even reach the alveoli of the inner lungs, these results stress the potential direct human exposure to microplastic contamination via indoor air.

State of the Science on Emerging and Novel Poly- and Perfluoroalkyl Substances (PFASs) (II)

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Global transport of perfluoroalkyl acids via sea spray aerosol
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Findings suggest that the long-range atmospheric transport of perfluoroalkyl acids (PFAAs) may be substantial, but it is currently not known what the main sources of atmospheric PFAAs are or how the compounds are transported in the atmosphere. PFAAs are present throughout the world’s oceans, which are currently assumed to be the final “sink" of PFAAs in the environment. However, sea spray aerosols have been suggested as a potential vector for ocean-to-atmosphere transport of PFAAs. We have used a laboratory system to study water-to-air transfer of 11 PFAAs via sea spray aerosol (SSA). SSA was generated from artificial seawater spiked to a concentration of approximately 10 ng/L for each studied substance. We observed significant enrichment of all PFAAs relative to sodium in the SSA generated. The highest enrichment was observed in aerosols with aerodynamic diameter < 1.6 μm, which had aerosol PFAA concentrations up to 62000 times higher than the PFAA water concentrations in the chamber. In surface microlayer samples collected from the sea spray chamber, the enrichment of the substances investigated was orders of magnitude smaller than the enrichment observed in the aerosols. In experiments with mixtures of structural isomers, a lower contribution of branched PFAA isomers was observed in the surface microlayer in relation to the bulk water. However, no clear trend was observed for the comparison of structural isomers in SSA and bulk water. Using the measured enrichment factors of perfluoroctanoic acid and perfluorooctance sulfonic acid versus sodium we have estimated global annual emissions of these substances to the atmosphere via SSA as well as their global annual deposition to land areas. The total annual flux of PFOA and PFOS to the atmosphere via SSA

estimated in this study is comparable to or exceed previous emission estimates for other potential sources of these substances to air. About 3% of the PFOA and PFOS aerosolised as SSA is transported and deposited to land areas. Our experiments suggest that SSA may currently be an important source of these substances to the atmosphere and, over certain areas where SSA deposition is important, a significant source to terrestrial environments.

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TBD

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Are bioaccumulation factors useful for regulation of PFOS in aquatic ecosystems?
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Accounting for bioaccumulation in regulation is complex and different jurisdictions have adopted varying approaches. Perfluoroalkyl acids (PFAAs) are a group of chemicals that bioaccumulate, but by a different mechanism to the better-understood chlorinated pesticides. In particular, perfluorooctane sulfonate (PFOS) bioaccumulates in aquatic fauna, but apparently due to protein affinity, rather than lipophilicity. Franklin (2016) described limitations of measures of biomagnification for PFOS, calling into question the use of BAFs in a predictive way. Here we summarise BCFs and BAFs in organisms with gills, particularly those reviewed by Ding and Peijnenburg (2013) and Moermond et al. (2010). The summarised BAFs varied over three orders of magnitude. Trophic level did not appear to be a good indicator of the variability, but there was a suggestion that sediments play a part in bioaccumulation of PFOS, as previously observed by Martin et al. (2004). The variability in BAFs and lack of clear understanding of the mechanism controlling bioaccumulation hinder the use of these metrics for regulatory purposes. Examples of two different regulatory approaches are provided to highlight issues with using BAFs to derive conservative criteria. Moermond et al. (2010) calculated water-based criteria for protection of birds and mammals by using BCFs and BMFs to extrapolate from toxicity thresholds to a concentration in water. In order to derive a conservative guideline value using this top down approach, it is appropriate to use a high estimate of BCF and BMF. Environment and Climate Change Canada [7] used a BAF to extrapolate from a water concentration, to a concentration in fish tissue that would be protective of the fish. Using this bottom up approach, it would be desirable to use a low estimate of BAF in order to derive a conservative guideline value. Although the approaches from RIVM and ECCC aim to protect different values, the need to select either a high or a low estimate of bioaccumulation is interesting and worthy of discussion. Both the large variability in bioaccumulation metrics for PFOS in aquatic organisms, and the uncertainty in mechanisms controlling bioaccumulation, suggest that factors such as BAFs and BCFs need careful consideration when deriving aquatic criteria. For some purposes, such as protection of fish from exposure by bioaccumulation, using tissue-based toxicity values will prove useful if more data become available from future testing.

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Prenatal exposure to PFOS and PFOA in a Catalan pregnant women cohort.
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The perfluoroalkylated substances (PFASs) perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been widely studied for their constant use in recent decades and their persistency, bioaccumulation and toxic properties. The aim of this study was to assess the prenatal exposure to PFOA and PFOS from a cohort of pregnant women in Reus (Catalonia, Spain). These chemicals were biomonitored from maternal blood during the first trimester of pregnancy, at delivery, and also from cord blood. Dietary exposure to PFOA and PFOS were determined using food frequency questionnaires and water intake, as well as the levels of these compounds previously analysed in foodstuff of Catalonia. In addition, the exposure levels of PFOS and PFOA were determined in indoor dust and air, using activity profiles of women. Finally, a physiological-based pharmacokinetic (PBPK) model was applied in order to: 1) establish the prenatal exposure of the fetus/child, and 2) evaluate the adjustment of exposure assessment (dietary and environmental) and biomonitoring results. Mean plasma levels were 0.45, 0.13 and 0.12 ng/mL for PFOA and 2.93, 2.21, and 1.17 ng/mL for PFOS at the first trimester, at delivery, and in cord plasma, respectively. Transplacental transferences of PFOS and PFOA of around 70% and 60%, respectively, regarding maternal levels at first trimester were observed. Similarly to previous studies, a temporal decrease trend in PFOA and PFOS blood levels were noticed, when the current results were compared with those of studies performed ten years ago in the same area. Dietary intake is the main route in our cohort. Fish and seafood are the main dietary contributors in this cohort of pregnant women. Maternal age (lower levels in younger mothers), parity (lower PFASs levels in women with previous children), and maternal country of birth (higher levels of PFASs in Spanish mothers) are determinants of the levels of PFASs, especially PFOA. Probabilistic calculations of fetal exposure were estimated by forward internal dosimetry using physiologically based pharmacokinetic (PBPK) model, and Monte-Carlo simulation. The physiological and chemical-specific parameters were adapted from the adult human model and modified for the fetuses and mothers, as a function of the gestational period. This is an important issue to establish the exposure in critical windows periods such as fetal development to PFASs, but also to other endocrine disrupting chemicals.

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PFAS research within the US Environmental Protection Agency: Results to date and future direction
A. Gillespie, U.S. Environmental Protection Agency / Atlantic Ecology Division
Per- and polyfluoroalkyl substances (PFASs) are a growing concern among many communities in the US due to increasing reports of potential human exposures. The science required to protect public health and the environment from PFAS exposure cuts across many applications and disciplines. The risk assessment/risk management paradigm provides a useful means to assess the state of the science available for informing decisions, and to identify gaps in knowledge needed to support risk management. US EPA's initial scoping of information available for assessing and managing PFAS risks revealed deficiencies in all key areas of the risk paradigm including hazard and toxicity (there are likely hundreds of PFAS in the US environment, and most lack sufficient suitable toxicity information for informing understanding of the potential for human or ecological effects); exposure (information about different PFAS sources, fate and transport, and human and ecological exposure is sparse, both spatially and temporally); and suitable treatment and remediation approaches (there is little information on effective methods and costs for treating or removing different PFAS from drinking water, groundwater, wastewater, air, soils, and sediments). US EPA is conducting an integrated set of research activities aimed at filling gaps in our current ability to conduct scientifically rigorous risk assessment and risk management activities. This research program is designed to address these data gaps and enable stakeholders to begin making effective decisions for identifying and mitigating risk from PFAS in the environment. Many different entities have an interest in – and are actively conducting – research to address PFAS, and so there is a substantial opportunity to advance PFAS science by improved coordination and collaboration. The purpose of this presentation is (1) to provide an overview of US EPA's overarching PFAS research program, with a focus on recent advances and ongoing activities, and (2) look for opportunities to collaborate with other organizations to advance the science needed to understand and manage risk from PFASs.

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Poster spotlight

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Contaminated Sediments: an Understudied Environmental Compartment (II)

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Toxic floods - Impacts of remobilized endocrine disruptors from sediments in rainbow trout (Oncorhynchus mykiss)
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Studies all around Europe have demonstated using *in-vitro* biotests that endocrine-disrupting chemicals (EDCs) can accumulate at high concentrations in river sediments ranging from 0.02 to 55 ng estradiol equivlanents per gram. Even small concentrations of EDCs can significantly interfere with the sexual development of fish, leading to the feminization of male fish and thereby adversely affect the reproductive success of whole populations. However, remobilization of sediment-bound EDCs e.g. due to bioturbation or re-suspension during flooding and associated effects on aquatic wildlife as fish is yet poorly understood. Therefore, the main objective of this study was to evaluate the risks of sediment-bound EDCs during flood events regarding a) whether they might become bioavailable to fish, i.e. rainbow trout (*Oncorhynchus mykiss*), when sediments are resuspended and b) if uptake consequently leads to negative effects in those organisms. Juvenile

rainbow trout were exposed over 21 days in groups of 24 individuals to six different treatments including 1) municipal tap water as water control; 2) sediment from Ehrenbreitstein (Rhine river, Germany) which shows low contaminant concentrations; 3) sediment from the Luppe river (Germany) highly contaminated with EDCs; and 4-6) three dilutions of Luppe sediment with Ehrenbreitstein sediment: 1:2, 1:4, and 1:8. Sediment (5 g/L dry weight) was constantly re-suspended using submersible pumps. Nonylphenol (NP, water control: 6 ng/L; Luppe: 1050 ng/L) and estrone (water control: 0.1 ng/L; Luppe: 7 ng/L) were detected by means of passive sampling (Chemcatcher) and subsequent LC MS/MS analysis. Moreover, high concentrations of NP were detected by LC MS/MS in bile samples ranging from 0.5 $\mu g/mL_{bile}$ (water control) up to 984 $\mu g/mL_{bile}$ (Luppe), whereas NP concentrations in blood samples were about 1000- fold lower. Total estrogenic activity identified by the yeast estrogen screen in bile of fish exposed to Luppe sediment was positively related to bile NP concentrations. Vitellogenin (Vtg) content in mucus samples of trout after 21 days of exposure to Luppe sediment was significantly higher as before exposure. Our findings demonstrate that sediment bound EDCs were remobilized, bioavailable and readily taken up by fish. Adverse effects are likely based on the induction of Vtg. Currently conducted studies on changes of estrogen-responsive genes will help to evaluate how pronounced these effects are.

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Are Diffusive Gradients in Thin Films (DGTs) useful predictors of the ecological effects of Cu in the field?

S.C. Udochi, A. Grant, University of East Anglia / School of Environmental Sciences

For its ability to measure the concentrations of free metal ions and labile complexes which may be relevant to biota uptake, Diffusive Gradients in Thin Films (DGT) has been projected as a useful tool to predict metal bioavailability and toxicity in aquatic systems. However, most studies establishing this relationship to date have relied on spiked sediments and/or measured metal bioaccumulation, which may not reflect ecological effects occuring in actual field sites. In this study, we extended the use of DGT to naturally-contaminated field sediments, and compared its predictive capability with traditional metal measurements including total metals, dilute-acid extractable metals, and measures based on the Equilibrium Partitioning Model (EqP). We deployed the DGT probes along a Cu contamination gradient in the Fal and Hayle estuaries, South West England, and assessed ecological effects using the pollution-induced tolerance of nematode communities as an endpoint. Total concentrations, extractable metals, and traditional EqP normalisations poorly predicted effects of Cu to the nematode communities at our sites. Conversely, the DGT-concentrations were correlated with observed effects, as well as porewater metal concentrations previously determined at the sites. DGT-Cu measured in the overlying water, 1-2 cm above the Sediment-Water Interphase (SWI), appeared to be more strongly related with effects than Cu in the surface sediments (1-2 cm below SWI). Although our results corroborate previous laboratory studies performed with spiked sediments, we note the relatively low dynamic range of DGT-concentrations measured in the field. This narrow range could considerably limit the applicability of DGT in the assessment of moderately-contaminated sites where effects might be occuring, thus challenging the usefulness of the technique in deriving sediment guideline values. Our work confirms the importance of field studies in ecotoxicology, highlighting the shortcomings of laboratory assays in creating realistic exposure conditions. We conclude that DGT, within its current use, is at best suited for assessing the relative bioavailability of dissolved metals between given sites.

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Effects of Oil Sands Bitumen Exposure in Aquatic Ecosystems: An Integrated Ecotoxicological Approach

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The Athabasca oil sands deposits in northern Alberta, Canada, are a naturally occurring mixture of bitumen, sand, clay and other minerals. Bitumen is a heavy and extremely viscous oil, which after mined and subsequently refined produces gasoline, diesel and other hydrocarbon-based products. Moreover, the naturally occurring Athabasca Oil sands deposits are a source of both physical and chemical stressors to local rivers that flow through the deposit. Physical stress to aquatic biota from natural bitumen results from hillslope erosion processes and slumping of material into the rivers, while chemical stress arises from bitumen-derived contaminants entering the waters. To fully understand the cumulative ecological effects of oil sands mining activities to aquatic ecosystem, in terms of water quality and associated biological structure and function, there is a need to previously evaluate the effects of naturally occurring bitumen in the aquatic environment. For that, a series of inter-related laboratory ecotoxicological assays were conducted using benthic and pelagic aquatic invertebrates exposed to oil sands material collected from four different sources in regional rivers. Organisms were sensitive to the presence of oil sands elutriates, especially to elutriates generated from bitumen collected in the banks of the Steepbank and Ells river, with effects on the life traits of *Daphnia magna*, *Physa acuta, Vibrio fischeri,* and *Dugesia tigrina*. When bitumen was mixed with sediment, it was also toxic to *Chironomus riparius,* which responded negatively bitumen samples with more bitumen content in sediments with a decrease in their body size, a delay on the emergence time, reduced total emergence and a decrease in the weight of imagoes. On a more complex approach, bitumen negatively affected natural communities tested in a mesocosms experiment by reducing the number of *Ephemera* sp. and *Chironomus* sp. that feed on fine sediment particles. The use of solid and elutriates bitumen samples, combined with a suite of representative species and different experiments provides a comprehensive and holistic approach to assess effects of oil sands materials arising from bank erosion-related processes, emphasising the need to discriminate natural processes from mining-related activities of surface and groundwater contamination in oil sands areas.

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PCB fate and transport at the Lower Ottawa River (USA): A seven-year pre-/post-dredging analysis using sediment, passive samplers, aquatic invertebrates, and riparian spiders

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In 2010, environmental dredging to remediate sediments contaminated with PCBs, PAHs, and lead was performed at select site locations in the Lower Ottawa River, a portion of the Maumee River Area of Concern (Ohio, USA), in 2010 to remediate. Prior to dredging, we designed a multi-metric study to assess contaminant fate and transport, ecosystem response, and recovery using a multiple lines of evidence approach. We measured PCB concentrations in surface sediment, passive samplers deployed in the water column, aquatic invertebrates, and spiders from 2009-2013 & 2015. Site-specific PCB concentrations were significantly and positively correlated among all metrics across all years except for sediment. Sediment concentrations at remediated and non-remediated sites were highly variable throughout the study period and were unreliable indicators of PCB concentrations in biota. PCB concentrations in aquatic invertebrates were highly correlated with concentrations measured in passive samplers, and in turn, were strongly predictive of concentrations in riparian spiders that are specialized predators of adult aquatic insects. All of these measures of PCB uptake, accumulation, and trophic transfer declined by 2015, indicating that the dredging remedy reduced PCB concentrations in biota to pre-remedy concentrations or below.

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The role of bioturbation activity in species specific sensitivity of benthic invertebrates to contaminated sediments

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Benthic invertebrate bioturbation activity enhances resuspension of chemicals into the water column, thus exposing sediment dwelling organisms to harmful substances locked up in the sediment. Yet, benthic organisms differ in bioturbation activity, which may influence their sensitivity to contaminated sediments, as higher burrowing activities increase the rate at which sediment associated chemicals are released. Since experimental evidence is still lacking the aim of the present study was to quantify the influence of benthic invertebrate bioturbation activity on their sensitivity to contaminated sediments. To this purpose intact sediment cores were collected at locations identified as ‘chemical hot-spots’ and subjected to whole sediment bioassays with six benthic invertebrates differing in bioturbation rate: the non-biting midge *Chironomus riparius* (low), the worm *Limnodrillus hoffmeisteri* (low), the amphipod *Gammarus pulex* (intermediate), the isopod *Asellus aquaticus* (high), the mayfly *Ephemera danica* and the caddisfly *Sericostoma personatum* (both with unknown bioturbation activity). The bioturbation activity of the selected benthic invertebrates was determined by measuring turbidity of the overlaying water. The three test species for which the bioassays were succesful showed the lowest survival on the agricultural sediment, that also affected isopod growth most. Yet, exposure to the other sediments also caused reduced isopod growth Bioturbation activity of *G. pullex* was significantly higher than that of the other three invertebrates. *A.aquaticus* and *C. riparius* showed similar bioturbation activity. *L. hoffmeisteri* showed the lowest bioturbation activity. Employing these bioassays allowed ranking of contaminated sediments based on biological responses rather than on the absence of presence of compounds. The species specific responses to the most contaminated sediment could however not be related to their bioturbation activity. When comparing the survival of the different test species, the insects *C.*

riparius and *S. personatum* suffered from higher mortality than the crustacean. Hence, the contamination of the agricultural location affected the insects most, due to presence of the insecticide chlorpyriphos in the sediment. It is concluded that the presence of compounds with a specific mode of action (insecticides) outweighs the influence of bioturbation activity on species specific responses to contaminated sediments.

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Paleoecotoxicology: Assessing the Toxicity of Lake Sediments Impacted by Legacy Gold Mining in Yellowknife, NT, Canada
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Natural resource extraction has supported the development of Canada's far north, but has also resulted in environmental contamination at some operations. Such is the case in Yellowknife Northwest Territories, where two now abandoned gold mines operated throughout the latter half of the 20th century 5km north of the city. Due to ore processing techniques, approximately 20000 tonnes of particulate arsenic trioxide was deposited onto the landscape surrounding the mine. Consequently, elevated surface water arsenic concentrations have been reported in lakes within 17km of the mine at present. Here we develop methods in paleoecotoxicology to characterize the full extent of historic metal(loid) contamination and assess the toxicity of these historic sediments. We examine the potential of analysing sediment archives with an ecotoxicological perspective to reconstruct missing biomonitoring data at historically contaminated sites. This study examines lake sediment cores from 21 lakes within 30km of the Yellowknife. Sediments were dated using radiometric dating techniques, and the toxicity of dated sediment horizons was determined using the novel application of microbial bioreporters and modified *Daphnia* toxicity testing techniques. Metal(loid) profile concentrations track a peak in concentration during the height of mining operations, which decreases with distance from the city. Initial assessments of bioavailability and acute *Daphnia* toxicity indicate that arsenic in porewater is 50-90% bioavailable, while sediments deposited during the time of mining operations decrease *Daphnia* survivorship. These results indicate that lake sediment archives can be used to reconstruct missing biomonitoring data in sites of legacy anthropogenic influence. Additionally, these results highlight the importance of applying techniques in paleoecotoxicology in cases of legacy contamination, and suggest that aquatic ecosystems continue to show lingering contamination from past gold mining activities.

Bridging the Gap: Maximizing the Role of Mechanistic Approaches (Including Omics) for Better Chemical Safety Decisions Across Humans and Ecosystems (II)

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Using transcriptomics to explain differential sensitivity to pesticide exposure: towards using omics to predict toxicology
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An *in silico* tool comparing omics to 'flag' sensitive species would improve risk assessment for untested species. To achieve this, we need to know how toxicokinetics (TKs) and toxicodynamics (TDs) contribute to differential species sensitivity (DSS). Therefore, we investigated the TKs and TDs underlying toxicity in earthworms exposed to the pesticides chlorpyrifos and imidacloprid, that both inhibit neuronal synaptic function by targeting Acetylcholinesterases (AChEs) and Nicotinic Acetylcholine receptors (nACHRs) respectively. Our analyses support the hypotheses that TDs drive the ~300x lower sensitivity of earthworms, relative to hexapods, to chlorpyrifos. For example, we find that a relatively moderate Cholinesterase (ChE) activity in the gut is the result of highly expressed but 'low-activity' ChEs. The inhibition of these ChEs by chlorpyrifos suggest earthworms have an underappreciated chlorpyrifos 'sink' that is unavailable to most hexapods, that generally possess only two neuronal ChEs. Earthworms are also ~100x less sensitive to imidacloprid than hexapods and, within earthworms, the Megascolecid, *Amynthas gracilis,* is ~30x more sensitive than Lumbricids, a difference that our comparisons suggest cannot be accounted for by differential TKs. The major TD target for imidacloprid is the synaptic nACHR-ion channels. In addition to nAChRs, the transcriptomics reveal that, unlike most hexapods, earthworms express high levels of Acetylcholine Binding Proteins (AChBPs), which are predicted to bind imidacloprid but lack an ion channel. Expression patterns suggest AChBPs have a nervous system function but previous analyses indicate they do not have a synaptic role. We hypothesise that AChBPs possess a moderately less critical (non-synaptic) function and act as a protective 'sink' preventing imidacloprid reaching the synapse. Furthermore, unlike the sensitive *A. gracilis*,the Lumbricids deploy a considerable portion of their AChBPs to non-neural tissues, perhaps offering greater protection of the neuronal functions mediated by the nAChR/AChBP targets. In summary, if the functions of nACHRs are more critical than neuronal-AChBPs, which are, in turn, greater than non-neuronal AChBPs, the expression of imidacloprid targets can arguably account for the observed DSS. Combined, these analyses suggest transcriptomics can aid the characterisation of TD drivers of DSS for pesticides and adds weight to the possibly of using omics to 'flag' sensitive species.

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Linking molecular response and DEB modelling approaches
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A working group at the National Institute for Mathematical and Biological Synthesis (NIMBioS) explored the feasibility of integrating 2 complementary approaches relevant to ecological risk assessment. Adverse outcome pathway (AOP) models provide "bottom-up" mechanisms to predict specific toxicological effects that could affect an individual's ability to grow, reproduce, and/or survive from a molecular initiating event. Dynamic energy budget (DEB) models offer a "top-down" approach that reverse engineers stressor effects on growth, reproduction, and/or survival into modular characterizations related to the acquisition and processing of energy resources. Thus, AOP models quantify linkages between measurable molecular, cellular, or organ-level events, but they do not offer an explicit route to integratively characterize stressor effects at higher levels of organization. While DEB models provide the inherent basis to link effects on individuals to those at the population and ecosystem levels, their use of abstract variables obscures mechanistic connections to suborganismal biology. To take advantage of both approaches, we developed a conceptual model to link DEB and AOP models by interpreting AOP key events as measures of damage-inducing processes affecting DEB variables and rates. To establish whether our approach has merit we developed a comprehensive molecular dataset based on *Chlamydomonas reinhardii*. Specifically, we generated metabolomics and lipidomics data as a result of exposure to 4 concentrations of Copper over 7 time points (0-145h). The design of the experiment was guided by principles required in both DEB modelling and OMICs data analysis. We analyse the molecular data using computational approaches to establish potential AOPs and ask the question whether the genes involved could further inform the DEB modelling procedures.

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Pharmacology-informed prediction of the environmental risk posed by mixtures of non-steroidal anti-inflammatory drugs (NSAIDs) to fish
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The presence of low concentrations of non-steroidal anti-inflammatory drugs (NSAIDs) in the aquatic environment has raised the concern that chronic exposure to these compounds may cause adverse effects in wild fish populations. This potential scenario has led to the inclusion of diclofenac in the European Union Watch List of emerging pollutants; however, there are currently over 30 NSAIDs on the market raising questions of mixture effects. Here we present an integrated pharmacology-informed framework that enables the prediction of potential adverse effects in fish exposed to a mixture of NSAIDs. This framework combines two mechanistic visions: network-centred and target-centred. Initially we generated an *in vitro* bioactivity network for a mixture of nine NSAIDs. This network, including over 140 nodes, was filtered using drug uptake and pharmacokinetic parameters to generate a more simple network displaying the biological activity expected at environmentally relevant levels of exposure. This was subsequently used to generate multi-organ target-phenotype anchoring predictions, specific for zebrafish, to identify the most likely phenotypic effects that might occur under the considered exposure scenario. This network-based approach was successively combined with a more traditional target-centred approach based on the pharmacological hypothesis that the sustained inhibition of NSAIDs primary targets - cyclooxygenase (COX) 1 and 2 – in healthy tissues is the key driver of toxicity. We developed a quantitative Adverse Outcome Pathway-informed model that incorporates both pharmacokinetic and pharmacodynamic aspects of NSAIDs toxicology. This dynamic visual model enables a rapid assessment of the risk posed by environmental levels of NSAIDs, and their potential to trigger multi-scale adverse effects. Although total COX inhibition activity in the plasma of wild fish may be sufficient to trigger COX IC50s, the mean effect concentrations for all 10 phenotypic endpoints are above predicted environmental levels. Only 9% of the effect concentration data points were within predicted environmental levels, residing within endpoints for reproduction, immunomodulation, male testosterone, and plasma prostaglandin. We anticipate that the integration of these mechanistic approaches will provide a useful predictive tool to support the implementation of effective NSAIDs ecopharmacovigilance strategies; facilitating the regulatory interpretation of past and future toxicity data.

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SKIPS: Stochastic Knowledge-based Interaction Pathway Simulator
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The field of toxicology suffers from a lack of predictive models and mathematical frameworks that link molecular processes to whole organism responses. A major problem is that measurements made from molecular level assays are inherently noisy, and such variance injects doubt into organism level outcomes linked to them. Even worse, whole organism measurements are often reported using low numbers of replicates, which may be sufficient to test for an effect (toxic/non-toxic), but are variable enough that deterministic models built from these data suffer from only marginal predictive fidelity. Given that experimental data is often quite variable, predictive models should aim to incorporate noise and uncertainty—not ignore it. Here we describe such an approach, termed the Stochastic Knowledge-based Interaction Pathway Simulator (SKIPS), which thrives on datasets that exhibit a broad range of measurement variation in both the inputs (e.g., molecular exposure assays) and endpoint measurements that span increasing levels of biological organization. Existing approaches to modeling noisy datasets shoehorn variation into deterministic models by adding a "noise term" (e.g., stochastic differential equations), but this method requires users to make broad, ad hoc simplifying assumptions regarding the type of noise (e.g., white or colored noise), and may therefore not effectively capture the statistics of the experimental data with low enough error to establish confident predictions of toxic effects. In contrast, SKIPS shortcuts a formal stochastic simulation by modeling the mean response at each biological level around which conditional Monte Carlo sampling is used to generate variation statistically consistent with training data. A conditional probability distribution associated with a biological response can thus be reconstructed using this method for certain input values. We demonstrate the utility of SKIPS via a reproduction-related adverse outcome pathway in the adult female fathead minnow (*Pimephales promelas*). SKIPS correctly captured the NOEC, LOEC, and point of departure (POD) measurements impacting average fecundity for chronic trenbolone bexposures. This success highlights the potential for SKIPS to support rapid screening efforts in toxicology or to enable decision making in applications where predicting chemical risks and hazards is important.

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Vision of a near future: bridging the Human Health - Environment divide. Toward an integrated strategy to understand mechanisms across species for chemical safety assessment

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There is a growing recognition that mechanistic approaches to understanding cross species target and pathway homology provide significant opportunities in risk assessment (RA) for both Human Health (HH) and environmental safety. Specifically, it has been recognized that a more comprehensive and reliable understanding of pathways will better enable the extrapolation of potential adverse impacts across species. Ultimately, this would allow a mutual exploitation for HH and Ecotoxicological RA to coherently and more efficiently characterize overall hazard. Given the broad inter-species differences occurring at multiple levels of biological organization, there is unlikely to be a "one-size-fits-all" solution that can effectively characterize species similarities / dissimilarities completely. However, there are some pragmatic first steps that can be taken using emerging and developing technologies (including high-throughput molecular techniques). Thus, there is a need for new approaches to identify, characterise and (ideally) quantify those differences and how mechanisms of action may differ across species. Motivated by these questions, Unilever sponsored a workshop to discuss how we can make better use of existing data and how to efficiently generate new data to derive mechanistic understanding between humans and environmentally relevant species and ultimately aim at more efficient, holistic (HH and Environment) chemical safety decisions. Experts from tripartite stakeholders (industry, academia and governmental agencies encompassing both human health and environment) were brought together to discuss this concept. This workshop highlights a common will to progress ever more to mechanistically-based data driven animal-free chemical safety assessments. The discussion addressed current gaps and key challenges, proposed solutions and identified research priorities for the future. Overall, the participants recognize that there is no silver bullet and no single fit-for-all approach which would provide us with all answers, but also acknowledged we have now the incentive, tools and information available to start developing this concept, maximizing the advantages of the different data sources to improve confidence and efficiency of RA.

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A framework to demonstrate the applicability of omics in human health and environmental risk assessments

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In recent years there has been a paradigm shift towards gaining more mechanistic toxicological insights in risk assessments. The drivers for this shift are a desire to move away from the animal testing requirements of conventional risk assessment as well as to move towards more transparent/scientifically valid approaches to toxicology [1]. Whilst advances in technology have resulted in an explosion of omics data being generated across multiple species, there is little evidence of it being used in chemical risk assessment or for making risk based decisions. One of the main perceived weaknesses of making decisions from genomics data is the lack of standardised approaches for method development, data analysis, data interpretation and validation. The approaches are also criticised as the observed effects are often not linked to a measurable apical endpoint in the species of interest or that an adverse effect is observed in a cell model without being able to see if those effects are likely to cascade down to organism effects. The use of omics data offers multiple potential benefits. Firstly, by looking at fingerprints of molecular perturbations following exposure to a toxicant, hazard identification can become possible by establishing a mechanism of action (MechoA). By looking at patterns of responses to compounds in well standardised systems, read-across may become possible. In addition, as we learn more about omics responses to different MechoAs, we will be able to design new and relevant biomarkers in the future for screening new chemicals. There is an understanding that we cannot test every ingredient on every organism, therefore, one of the greatest advantages to the use of omics data is that it allows us to look across species to identify areas of commonality or concern across human health and environmental risk assessment and to maximise opportunities for inferring mechanisms from one species/ test results to another. The aims of this work are to demonstrate that although we might not be ready to perform human health or environmental risk assessments based solely on genomics data, we can start including it alongside conventional data. This can be used as part of a WoE approach to gain better mechanistic insight. Here we present a proposed framework to demonstrate how new mechanistically derived data can be used to complement our current approaches to deliver a robust risk assessment applicable across both human health and the environment.

New Frontiers in Life Cycle Inventory Data Collection and Modelling (II)

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Methodology for the development of a hybrid regional supply and use model for the three Belgian regions

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Recently, environmental-extended multi-regional-input-output (EEMRIO) datasets have been developed enhancing the possibilities to assess environmental impacts of products, sectors and regions with economy-wide scope. However, compared to these developments, subnational EEMRIO datasets and applications develop at slower pace although the political demand for regional assessment is high. This assessment at regional level requires reliable and complete regional data inventories. However, there are still practical barriers to build such inventories, e.g. the access to and the compatibility of regional data are often restricted and the collection of new data is time-consuming. With this research, we want to demonstrate how such an inventory can be developed at regional level and propose a methodology to build regional hybrid supply and use tables (HSUTs). This model can perform material flow analysis and environmental impacts assessments in order to support resource management strategies. In this study we focus on the following methodological developments: increasing the spatial resolution, improving the product and sectors detail as well as measuring the products flows in physical units rather than monetary units. These developments of the inventory will be achieved through a combination of existing datasets. The available datasets for Belgium include: the national monetary and hybrid supply and use tables of EXIOBASE, the regional monetary SUTs (MSUTs) and available auxiliary data obtained from regional statistics such as: physical data on production and waste treatment data. We regionalize the Belgian HSUTs of EXIOBASE using the regional MSUTs and the auxiliary data at regional level.The regional hybrid model is the first attempt, to our knowledge of regionalizing EXIOBASE. If regional data are available, the developed methodology is applicable for another country covered in EXIOBASE and when regional data are available. The major shortcoming of the model is that by far not all needed data was readily available in physical units. To overcome the limitation, first estimations were made, which were then improved with a balancing procedure. But even though the model is data dependent, it is a flexible tool that offers the possibility to be updated when new data becomes available. <br clear="all" /> [1] "region" in MRIO refers to a country or a group of country. [2] Hybrid refers to the mixed-unit framework including: mass and energy (products) and monetary (services).

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Introducing the trade "supply mix" to improve regionalisation of Life Cycle Inventory for agricultural commodities
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One important challenge towards further regionalisation of life cycle assessments (LCAs) is to provide life cycle inventory (LCI) flows at a fine enough spatial resolution to reduce uncertainty of potential impacts. This challenge is particularly important for agri-food products given spatial variability in production. Here, we introduce the trade "supply mix" as the ensemble of elementary flows of an agricultural commodity for a given supply chain, which we apply to provide biome specific land occupation and transformation LCI for one tonne of Brazilian soy produced in 2017. We use the Transparency for Sustainable Economies information platform (Trase) which combines customs declarations, tax and shipping information to link municipality-level soy production to importing countries. This information is then connected to Brazilian biome boundaries and deforestation data to obtain biome specific land occupation and transformation elementary flows for soy destined to China, France, Germany and Finland. Results show different biome sources for soy imported into France and China compared to Germany and Finland. China and France showed greater land occupation in the Amazon with land transformation occurring in Amazon and Cerrado biomes. Germany and Finland's imported soy had a land occupation LCI with a greater proportion of Atlantic Forest compared to other biomes, with Finland, however, showing no land transformation LCI associated with soy. After calculating the biodiversity damage associated with soy exports, we noted that Finland had the largest land occupation damage, followed by China, France and Germany, while both China and France had similar land transformation biodiversity scores. Given the nature of the trade data and their spatial resolution, the trade supply mix could be used to calculate other elementary flows for agri-food products (e.g., water use, greenhouse gas emissions, fertilizer application) and can also be related to companies and organizations such as producers and traders. These elementary flows will be derived and made available through the Trase platform.

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Significance of pesticide emission modelling for assessing ecotoxicity impacts of agricultural systems
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With the increased global demand for agricultural products and the interrelated concerns on the environmental impacts, there is a need to have efficient tools to evaluate the environmental performance profiles of agricultural systems, to facilitate a move towards more sustainable production. Life Cycle Assessment (LCA) is widely applied to quantify potential impacts of products and systems along with their entire life cycles. One of the main challenges in assessing the environmental performance of agricultural systems in LCA is modelling emissions from pesticide use. Hence, this study aims to evaluate the effect of modelling choices to estimate pesticide emission fractions on the environmental impact profiles in a case study conducted on feed crops in Denmark (DK). This study followed the LCA methodology to evaluate the potential ecotoxicity impacts on freshwater ecosystems from pesticide use in DK's crop production. This analysis focuses on the evaluation and influence of pesticide application on the environmental impact profiles of maize, winter wheat, grass, spring barley, rapeseed and peas, testing the effects of the choice of the emission-modelling framework. Two types of local sensitivity tests were conducted, a scenario sensitivity analysis to test the effect of LCI modelling on the impact profile of the crops and a local sensitivity to input parameters for the reference scenario. Three different emission-modelling choices were tested; they differ on the underlining assumptions and data requirements. The median results for the resulting emission fractions vary by four orders of magnitude for the different models. Main aspects influencing impact results are the interface between inventory estimates and impact assessment, and the consideration of intermedia processes, such as crop growth development and pesticide application method. As showed neglecting such inter-media process, may entail overestimation of the emission fractions, and hence, application of the conservative default pesticide emission may lead to an overestimation of the potential toxicity impacts induced by application of pesticides in most agricultural LCA. Statistical differences were found in the impact results with two of emission model tested, thereby indicating the influence of modelling choices on ecotoxicity impact assessment. In conclusion, significant differences in emission estimates and related impacts were found between the scenarios for all crops.

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Simple N direct field emissions for agricultural LCA: towards a model adapted to contrasting climate and agricultural management
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Estimating nitrogen losses (in all its different forms: NH_3, NOx, NO_3, N_2O) from field nitrogen management (fertilisation, soil cover, *etc.*) is one of the main challenges in agricultural LCA. N losses are a major contributor to several LCA impact categories (*e.g.* climate change, eutrophication). LCA practitioners typically use the most accessible models, in terms of data demand and ease of use, such as emission factors or pre-defined model sets associated with databases. However, these models are predominantly representative of field crop conventional fertilisation by synthetic fertilisers. They are not well adapted to contrasting climates (from temperate, dry to tropical conditions) or contrasting agricultural systems (vegetable gardening, intercropping, perennials). Furthermore, they are not (or very little) sensitive to farming practices and cannot assess the effect of "eco-designed" farming systems or the particularity of organic fertilisers. After a review of the different N field emissions models used in LCA, we identified the potential of the INDIGO-N model for improving N emissions assessment in agricultural LCA. Without being a complex soil-plant dynamic model, INDIGO-N addresses effects of climatic and soil conditions, fertilisation in regard with the plant's needs, or some management practices. The goal of this work is to improve the INDIGO-N model to create a reference model for N losses in agricultural LCA. The objectives are multiple: ? improve the organic fertilisation assessment (with calculation of the mineral fertilisation equivalent of each input) ? assess a wide range of crops with different cultivation durations (field crops, vegetables, associations, perennials) ? integrate each crop into its crop sequence ? adapt the assessment to different climates (temperate, tropical, sub-tropical, dry) ? take into account the plant's needs and a range of agricultural practices (e.g. mitigation strategies, over-fertilisation). While being more complex than most of the simple N models used in LCA, INDIGO-N aims at being adapted to LCA constraints of feasibility, allowing to estimate the N losses for both average productions (*e.g.* a national average wheat) and specific eco-designed agricultural systems. An excel tool will be provided to calculate the N losses and the model will be implemented into MEANS-InOut, an LCI-generator software for agricultural LCA developed by INRA and CIRAD.

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Towards global LCI data on urban wastewater discharges
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We present version 3 of WW LCI, a model to calculate LCIs of urban wastewater discharges in 69 countries. It covers wastewater treatment at primary level (settling tank), secondary (biological treatment), tertiary (N/P removal, filtration, disinfection) and septic tanks. Sludge disposal includes composting, landfilling, incineration and agricultural use. We have improved the model at two levels: **1) Wastewater components**: the model was designed to cover modelling of chemicals in wastewater, where the individual chemicals must be identified, as done in environmental risk assessment. In WW LCI v3 we adapt the model to also support generic descriptors: chemical oxygen demand (COD), suspended solids (SS), toal N and total P. Also, the presence and fate of heavy metals is now supported too. The model is now capable of a complete coverage of wastewater components: organic matter, nutrients, organic micropollutants and metals. **2) Country coverage**: The database in WW LCI v3 has been extended from 56 to 69 countries (83% of the world's population). New variables by which every country is now described includes: - Methane emissions from open sewers, adjusted for the country's climate. - Electricity consumption in the wastewater treatment plants (WWTPs) as a function of plant capacity. - Wastewater infrastructure (sewers and WWTPs) is quantified for each country using five capacity classes. - Extent of anaerobic digestion of sludge and cogeneration of heat and electricity with biogas is established for each country. - The heat balance at the WWTP is calculated taking into account the country's climate. - Sludge landfilling includes uncontrolled landfills. In each country we define a controlled/uncontrolled landfill mix. - Emissions of pollutants are specified as a combination of discharges to the sea, freshwater and groundwater. We present results for the 69 countries in the database, for discharging 1 m3 wastewater with (in mg/L): 500 COD, 250 SS, 30 N and 6 P. Greenhouse-gas emissions per m3 range from 0.3 kg CO2-eq in Japan to 1.7 kg CO2-eq in Bangladesh. Results can be calculated for any other impact category, using LCA software. This is to our knowledge the most complete LCI tool for urban wastewater. It is flexible, allowing the user to either rely on chemical-specific assessments, specific country conditions, or on generic measures (COD, N, P, SS) and average country conditions. We expect the country database will keep expanding to achieve a global coverage.

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LCA of urban water systems: STOIC, a dynamic LCI model linking human activities and wet-weather pollutant discharges
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With unprecedented urban growth at the planetary scale, urban wastewater systems (UWS) are faced with the operational challenge of managing ever increasing and polluted stormwater to mitigate impairments of the quality of

aquatic ecosystems. However, to which extent do wet-weather control strategies actually help reduce overall environmental impacts? With the general aim of assessing in a whole-systems perspective to improve the environmental assessment of UWS with Life Cycle Assessment (LCA), by including impacts from operation and infrastructure, and impacts from treated pollutants in discharges, the objective of this work is to answer the question: "Is it possible to perform the LCA of Urban Wastewater Systems that captures the temporal variability of water discharges in an urban catchment?". This work describes a site-dependent framework to estimate for life cycle inventory (LCI) purposes, intermittent discharges (ID) from UWS with consideration for their important spatiotemporal variability at the urban catchment scale. The model (named STOIC, as an abbreviation for "STOrmwater Inventory Conceptualization") satisfies requirements on inventory data quality to support sufficiently accurate modelling of IDs and conceptualizes stormwater processes occurring within an urban catchment and its UWS. These stormwater processes include the generation of water pollutants from nonpoint source emissions to the routing and distribution of pollution in the UWS components, resulting in spatiotemporally differentiated LCIs for water pollutants within an urban catchment. The STOIC framework is applied to a UWS at sub-catchment scale to assess global performances of different control strategies for IDs in terms of environmental impacts in a real-world case study in Bordeaux, southwest France. This demonstrates the interest and applicability of an extended environmental assessment which considers the temporal variability of UWS discharges from an urban sub-catchment.

Incorporating Ecosystem Functioning into Environmental Quality Assessment

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Linking ecotoxicological risks and ecosystem functioning
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In response to environmental quality deterioration, policies, like the EU Water Framework Directive (WFD), have been adopted that aim at achieving a good chemical and ecological status of water bodies. According to the EU WFD, monitoring strategies should include the assessment of both ecosystem structure and functioning in the determination of the ecological status. However, in practice the monitoring of surface waters often only encompasses structural measurements related to physicochemical parameters and biological community composition, while ecosystem functioning, despite its relevance, is largely overlooked. Therefore, this study aimed to investigate the relationship between contamination driven ecotoxicological risks and ecosystem functioning in Dutch surface waters. To this end, measurements of toxic pressure and decomposition were conducted at 19 surface water locations with different pollution sources. Toxic pressure was assessed by employing a bioassay battery of *in situ*, *in vivo* and *in vitro* bioassays. Decomposition by microbes and invertebrates was measured *in situ*. The present abstract discusses the preliminary results of the bacterial inhibition bioassay and microbial decomposition rates, awaiting promising results of further analyses. Bacterial inhibition at WWTP sites, caused by polar compounds, coincided with elevated microbial decomposition rates at these locations. These results suggest that WWTP locations accommodate a highly adapted microbial community that is capable of organic matter decomposition at a rate exceeding that of all other investigated locations, despite the observed toxic pressure to bacteria. The here presented observations shed new light on the role of ecosystem functional parameters such as decomposition in the determination of surface water quality. Functional parameters can be included in surface water quality monitoring, but a better understanding and rationale behind the attribution of certain water quality scores based on functional parameters, and their relationship to observed toxic pressure is still lacking. It is concluded that only by combining environmental chemistry (compound concentrations), toxicology (adverse effects of environmental samples) and ecology (ecosystem functioning), an integrated cause-effect oriented water quality assessment can be achieved.

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Meta-analysis of glyphosate contamination in surface waters and dissipation by biofilms
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One consequence of the intensive use of glyphosate is the contamination of rivers by the active substance and its metabolites aminomethyl phosphonic acid (AMPA) and sarcosine, inducing river eutrophication. Biofilms are the predominant lifestyle for microorganisms in rivers, providing pivotal roles in ecosystem functioning and pollutant removal. The persistence of glyphosate in these ecosystems is suspected to be mostly influenced by microbial biodegradation processes. The present study aimed to investigate the tripartite relationship among biofilms, phosphorus and glyphosate in rivers. The first part consists of a co-occurrence analysis among glyphosate, AMPA and phosphorus using an extensive dataset of measurements (n = 56198) from French surface waters between 2013 and 2017. The second part investigated the capacity of natural river biofilms to dissipate glyphosate, depending on phosphorus availability and the exposure history of the biofilm, in a microcosm study. A strong co-occurrence among glyphosate, AMPA and phosphorus was found in surface waters. More than two-thirds of samples contained phosphorous with glyphosate, AMPA or both compounds. Seasonal fluctuations in glyphosate, AMPA and phosphorus concentrations were correlated, peaking in spring/summer shortly after pesticide spreading. Laboratory experiments revealed that natural river biofilms can degrade glyphosate. However, phosphorus availability negatively influenced the biodegradation of glyphosate and induced the accumulation of AMPA in water. An increase in alkaline phosphatase activity and phosphorus uptake was observed in glyphosate-degrading biofilms, evidencing the tight link between phosphorus limitation and glyphosate degradation by biofilms. The results of the present study show that phosphorus not only is a key driver of river eutrophication but also can reduce complete glyphosate degradation by biofilms and favour the accumulation of AMPA in river water. The predominant role of biofilms and the trophic status of rivers must therefore be considered in order to better assess the fate and persistence of glyphosate.

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Using stable isotopes (δ13C and δ15N) together with functional microbial, plant and soil parameters to uncover effects on soil nutrient cycling in polluted fields
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The microbial degradation and transformation of complex organic material (e.g. litter, dead plants) towards smaller inorganic compounds and nutrients that can be taken up by plants, is a crucial soil process that determines soil fertility and productivity of terrestrial ecosystems. Effects of pollutants on microbial functioning have been mostly studied under controlled (lab) or semi-controlled field conditions, but there is a need for more field studies and non-disruptive ways to study microbial functioning. Since there is not one golden parameter to study microbial nutrient cycling, we applied a combined approach using different functional microbial, soil and plant parameters together with stable isotope techniques. The measurement of microbial soil respiration (CO_2 released with the breakdown of organic compounds) is the most direct way to assess effects on microbial activity. To enable a field study, we add cane sugar (naturally isotopic labelled) to bypass root interference and stimulate microbial activity. In addition, shifts in C and N content and the natural isotopic abundance of ^{13}C and ^{15}N in plant, soil and microbial biomass provide a more time-integrated view on the nutrient cycling function and are not influenced by short-time fluctuations. So far we studied a lead (Pb) polluted shooting range, a copper (Cu) polluted wood impregnation site, and a lysimeter experiment. The results of the first field study suggested that our methods are useful in evaluation of nutrient cycling in field studies of polluted sites. A decreased microbial decomposition in high Pb polluted plots was suggested by a lower substrate-induced respiration of the cane sugar. Furthermore, from the combined results of soil, plant and microbial biomass C, $\delta^{13}C$, N and $\delta^{15}N$, we drafted conclusions about disturbance of N cycling as well as nutrient- and/or water stress to plants. Results from other sites are currently under evaluation and will be used as a comparison and to confirm usefulness of the methods. This approach remains challenging, because it is dependent on a suitable low or non-polluted reference soil in the same area. Furthermore, a discussion should be held on the definition of disturbance of nutrient cycling, e.g. which parameters, direction and rate of change. We show that a combination of functional microbial, plant and soil parameters, and the aid of stable isotope techniques, may help to detect changes in ecosystem functions in polluted sites.

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A trait driver ecological theory predicts complex responses of phytoplankton exposed to chemical stress
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Understanding the processes shaping the structures and functioning of ecosystem is key to assess their vulnerability and predict their development under stress. The trait-driver theory (TDT) was recently introduced to describe the relationship between organisms' characteristics (traits), their assembling in communities and the emerging ecological functions. In so, it mechanistically details the processes underpinning response capacity (RC), the efficiency of a system in maintaining functionality by changing structures when conditions change. Despite being a fundamental concept in resilience theory, no direct measurements of RC has been drawn from empirical observations, so far. Furthermore, a detailed experimental assessment of TDT predictions is missing too. We run in situ experiments with two distinct lake phytoplankton communities stressed for the first time with a

mixture of micropollutants at environmentally realistic concentrations. We drew TDT expectations on community responses using an individual-base model. We compared formally elaborated theoretical expectations with the results of the experiment and found that TDT successfully predicts changes in size distribution, functional group turn-over and functional responses of the two communities. We also provide empirical evidence and a measure of their RC by looking at the rate of change of phytoplankton traits and its correlation with production. An empirical assessment of ecosystem RC is presented here for the first time. RC emerges as a trait-based measure of community responses linked to the maintenance of ecological functions under environmental changes.

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Freshwater phytoplankton community response across different historical contamination backgrounds
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Long-term recurrent exposure caused by the herbicide runoff from fields can impose a strong selective pressure on natural freshwater phytoplankton communities, favoring more tolerant species (pollution induced community tolerance - PICT). Adaptation to pollutants occurs through physiological and evolutionary changes. However, acquiring tolerance may have a trade-off where the most tolerant species might not be the most productive. The aim of this study is to differentiate between the imprint of long-term adaptation vs. short-term acclimation of natural phytoplankton communities originating from different historical contamination backgrounds. We conducted a 2-phase controlled experiment with phytoplankton communities grown from sediment containing seed banks (biological archives) from 2 lakes: a near-pristine forested (Finnsjön) and an Isoproturon historically exposed agricultural catchment (Tåkern), in Sweden. In phase 1, phytoplankton communities from the 2 lakes were germinated for 2 weeks in presence and absence of a sub-lethal level of Isoproturon (12 μg/L). During phase 2, we exposed the germinated communities for a week to 4 Isoproturon concentrations (7, 12, 61, 92 μg/L) following total biovolume development, photosynthetic efficiency (maximum quantum yield of photosystem II, F_v/F_m), and community composition. Lakes differed in biomass development, photosynthetic efficiency and community composition. Tåkern showed higher biovolume in phases 1 and 2. However, in phase 2, when exposed to the highest herbicide levels, Tåkern biomass dropped significantly compared to Finnsjön. Shannon diversity index evidenced the presence of tolerant species in Tåkern, favoured at higher exposure levels. Generally, Tåkern had a better photosynthetic efficiency recovery than Finnsjön. In particular, in Finnsjön the presence of herbicide during germination had a detrimental effect on the recovery of photosynthetic yield at higher exposure levels, whereas beneficial effects were observed in Tåkern. Long-term adaptation favours species with better tolerance to chemical disturbance at the expense of the most productive one. Short-term acclimation had a positive noticeable effect only on the recovery of photosynthetic efficiency and were dependent of previous long-term exposure. The pristine lake showed no sign of acclimation but more efficiency in sustaining biomass production even at high exposure levels.

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Novel experimental approaches to study complex phytoplankton community responses to contaminant exposure
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Diffuse contamination by chemical compounds is a wide-spread phenomenon in freshwater ecosystems in densely populated areas. Little is known about the effects of chronic exposure on ecosystem structure and functioning. The complexity of natural systems prevents straightforward inferences from standard test results towards community level changes, in particular ecological processes and functions. During a 4yr research project on *Pollution and ecosystem adaptation to changes in the environment*, we developed and deployed successfully setups to study complex phytoplankton community responses. The first experiment mimicked chronic background exposure to different levels of 12 common PPCPs (pharmaceuticals and personal care products). Phytoplankton was collected from lakes, transferred to dialysis bags, spiked with PPCPs, and incubated *in situ* at the depth of maximum chlorophyll a concentration for three weeks. The development of the communities was documented every 4th day with respect to different structural and functional measures. Phytoplankton was growing well within the bags. The variation across replicates was low and allowed tracking the effects of PPCPs on communities' structures and performances reliably. A second experiment addressed the role of adaptation vs. acclimation in a phytoplankton response towards herbicide exposure. To this end, natural communities were germinated from sediments of two lakes with contrasting historic contamination, in the presence and absence of isoproturon. The resulting communities were then exposed to different isoproturon levels. Measured endpoints included growth rates, photosynthetic efficiency, biovolume accumulation, species composition and biodiversity. Phase I of this experiment yielded divers algal communities, with species compositions being distinct for each lake. Phytoplankton communities originating from the agricultural catchment lake performed better when being exposed to acutely toxic concentrations of isoproturon, compared to those originating from the forested catchment – irrespective of their germination conditions in the laboratory. For moving ecotoxicology more towards stress ecology it is essential to develop approaches that allow to test natural communities' responses under realistic scenarios and on relevant time scales. This is the only way to reveal the ecologically relevant long-term impact of contaminants in the environment, including its interference with biological processes and functions.

New Developments in the Science of vPvB and PBT Assessment (II)

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Bioconcentration of cationic surfactants in fish
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The bioconcentration of a series of methyl alkyl amines was studied in rainbow trout. The test chemicals included primary, secondary, tertiary and quaternary amines ranging in chain length from C9 to C16. In an initial set of experiments we explored the tissue distribution of the test chemicals in ?130 g rainbow trout. After 7 d of exposure to mixtures of the alkyl amines the fish were sacrificed, and samples of muscle, liver, gill, skin and skin mucus were taken for analysis. All of the alkyl amines except for the two quaternary amines were quantified in all of the 5 tissues, and it was concluded that all of these tissues must be included when assessing the bioconcentration of alkyl amines. In a second set of experiments we measured uptake and depuration of mixtures alkyl amines in ?11 g rainbow trout and determined bioconcentration factors (BCFs). The experiments were conducted at 2 different pHs (7.8 and 6.3), with an exposure phase of 14 d and a depuration phase of 28 d. The concentrations of the test chemicals in water were relatively constant during the exposure experiment, with relative standard deviations ranging from 2-9%. This shows that it is possible to overcome the experimental challenges to conducting BCF studies for these strongly sorbing chemicals. Extracts of whole fish homogenates are currently being analysed for the determination of the BCFs. The influence of the structure of the alkyl amines and the pH on the BCF will be presented and discussed.

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The Problem with Growth Correcting the BCF and BMF in Bioaccumulation Assessments & Possible Solutions
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The OECD 305 guidelines for conducting aqueous and dietary bioaccumulation tests include the determination of a growth-corrected bioconcentration factor and biomagnification factor. While a growth correction appears appropriate for assessing bioaccumulation in very slowly growing fish species, the method by which the growth correction is performed violates (i) the mass-balance assumption, on which the bioaccumulation model is based; and (ii) the bioenergetics model, which, although not specifically recognized in the bioaccumulation model, underlies a correct description of the bioaccumulation process. Violating the mass balance assumption produces implausible bioconcentration and biomagnification factors that poorly represent the biomagnification behaviour of a chemical in nature. In addition, the OECD 305 guidelines regarding growth correction do not correctly account for the error in the BCF and BMF values caused by the growth correction. This error affects the comparison of measured BCFs and BMFs to regulatory criteria. In this presentation, we (i) illustrate the theoretical rationale for why the growth correction of the BCF and BMF is incorrect; (ii) present the error in the BCF and BMF values as a result of incorrect growth correction for a range of chemicals; (iii) present methods for conducting growth corrections that respect mass balance and energy balance; and (iv) discuss the relevance of growth correction on the classification of chemicals as Bioaccumulative (B) or Very Bioaccumulative (vB) substances.

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Conduction of a freshwater trophic magnification study based on a comprehensive literature evaluation

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To assess chemicals for their environmental risk, one characteristic evaluated is the potential to bioaccumulate in organisms. Laboratory derived endpoints have been representing the gold standard. In the light of the 3R principle for animal studies (Reduce, Replace, Refine) and a need for more holistic consideration of bioaccumulation alternative methods are being investigated right now. One of these metrics is the trophic magnification factor (TMF). This endpoint integrates enrichment processes over a food web and is derived from field samples. TMF values are also applied in the context of the Water Framework directive to normalise chemical monitoring results to a common trophic level. The derivation of environmental quality standards for the 'secondary poisoning of predators' goal is another aspect TMF values are important for. Recent reviews show that manifold factors could influence the TMF, accordingly research should be conducted to evaluate the respective impacts. A comprehensive literature evaluation was conducted to identify the requirements for the performance of TMF studies, focusing on freshwater habitats. A sampling campaign which considered many of the findings was successfully performed at lake Templin, Potsdam, Germany. Passive sampling covering the entire sampling period was conducted and plankton samples of different fraction sizes and sampling dates were retrieved. Mussels were collected as representatives of primary consumers (2nd trophic level). Since larger invertebrates are absent in the pelagic food web of lake Templin, fish samples of small and juvenile specimens representing intermediate trophic levels were included. Considerably larger and older fish were collected to provide insight into the upper trophic levels. These fish were divided into filet and carcass samples to allow analyses of both, accumulation and tissue distribution. Furthermore, blank samples of plankton, mussels and fish (filet) were taken. Preparation was performed following ESB cryo-milling and storage standards. Enough sample material for the different trophic classes could be obtained allowing the analyses of the food web interactions. However, the sampling campaign also showed that not all requirements specified for TMF studies in the literature can be applied to any environment. In how far these or other aspects could potentially impact the TMF determination is part of ongoing questions that are currently under investigation using the retrieved samples.

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Predicted trophic magnification factors for cyclic volatile methylsiloxanes in selected real-world aquatic food-webs
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An important aspect of environmental risk assessment is to evaluate the bioaccumulation potential of hydrophobic cyclic volatile methylsiloxanes (cVMS) through dietary exposure pathways. The current study simulated the chemical transfers through real-world food-webs and predicted trophic magnification factors (TMFs) for octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5). We selected five aquatic food-webs (Lake Erie, USA; False Creek, Canada; Lake Ontario, USA/Canada; Oslofjord, Norway; Tokyo Bay, Japan) and conducted a TMF simulation by using the Multibox-AQUAWEB (MBAW) biomagnification model. In order to reduce uncertainty for the most influential input parameters, such as biotransformation rate constants (k_M) and dietary assimilation efficiencies (E_D), measured values were used in the model instead of estimated ones. For the selected food-webs, all predicted TMFs were shown to be below 1, indicating trophic dilution of the compounds through the dietary exposure pathways. The predictions were generally in agreement with field-measured TMFs for D4/D5. However, there remains some disagreement concerning D5 in some aquatic systems, where field TMFs >1 or < 1 are rather uncertain based on the size of confidence intervals. Thus, it is suggested to investigate whether the chemical transfers in some systems were solely based on the food-web structure or affected by other factors such as environmental conditions (e.g., concentration gradients downstream of wastewater treatment plants).

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Applying a Weight of Evidence Evaluation for Characterizing the Bioaccumulation Potential of PAHs in PBT Assessment
A.D. Redman, Exxon Mobil Biomedical Sciences, Inc. / Toxicology and Environment Science Division; C. Hughes, Ricardo Energy & Environment / Chemical Risk; Y. Verhaegen, E. Vaiopoulou, CONCAWE; T. Parkerton, ExxonMobil Biomedical Sciences Inc. / Toxicology & Environmental Science
In a general scientific approach, assessment of persistence (P), bioaccumulation (B), and toxicity (T) of chemicals is an important initial step in the characterization of chemical risks in the environment. The science underlying bioaccumulation assessments has evolved over the past 50 years, with the initial focus on limited classes of recalcitrant chemicals that are not broadly representative of chemicals currently in commerce. Recent advances in B science have proposed new methods for evaluating PBT properties. The main goal of these methods are to identify substances with the potential to biomagnify in foodwebs and thus increase potential exposures and associated risks to upper trophic levels, including humans. The present work builds on these recommendations to specifically evaluate the bioaccumulative potential of polyaromatic hydrocarbons (PAH). this presentation will discuss B assessment of PAHs using fish BCF data as well as higher tier B metrics such as BMF and TMF. The behavior of PAH will be contrasted with classic POPs

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Obstacles in Finding New POPs: Bioaccumulation and Toxicity
H. de Andrade, ETH Zurich / Department of Environmental Systems Science; M. Scheringer, ETH Zurich
The Stockholm Convention (SC) recognizes Persistent Organic Pollutants (POPs) in terms of their persistence (P), bioaccumulation (B), long-range transport potential (LRTP), and toxicity (T). To nominate a new POP, data on specific screening criteria are necessary, such as B and T. Here, we discuss important issues in finding appropriate data for B and T criteria set by the SC. To start a POP candidate nomination, potential POP candidates must be identified. For 9 potential POP candidates with high K_{ow} values, we collected POP-specific data collected from public sources and evaluated this information for data availability and quality. Missing data were then generated with property estimation methods. A large obstacle in the data collection and evaluation was lack of measured B and T values. The core problem was identified as poor consideration of solubility and timescale issues. From a thermodynamic point of view, the solubilization in water is low for high-K_{ow} chemicals. In addition, also uptake via gills or membranes is slow because of the high K_{ow}. To reach relevant concentrations where B occurs and toxic effects are observed, a longer timescale must be considered. The ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.11, and the OECD Test Guidelines (TG) 305 (B) and 203 (acute T) acknowledge issues in testing low-solubility substances. The current TG 305 explicitly mentions the use of aquatic dietary exposure for very hydrophobic chemicals in B tests, but in actual B tests, dietary exposure is often not considered. Regarding T, TG 203 does not mention dietary exposure and offers limited recommendations for the solubilization of hydrophobic compounds. Beyond the 9 substances considered here, many POP candidates have low to almost no solubilization in aquatic B and T test media. A better way to create aquatic exposure scenarios would be to simulate dietary uptake. The limitations described are a great barrier in a new POP nomination, because the SC prefers experimental data. POP candidates may be left behind if the requested data are not available and/or cannot be generated. It is imperative to develop better standard tests for dietary exposure that accommodate the unique properties of POP-like substances or enforce guideline recommendations. Overall, there is need for more standardized, routine-like applications of new methods. The SC should consider updating its criteria to resolve these issues in B and T assessments.

Communicating Science, Failures, Fortune and Risks to Improve the Value of Environmental Research to Society (II)

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Stakeholder engagement in environmental risk assessment
W.R. Munns, U.S. Environmental Protection Agency / Atlantic Ecology Division; H. Selck, Roskilde University / Dept Science and Environment; A. Rea, U.S. Environmental Protection Agency / Immediate Office of the Assistant Administrator
Active engagement with stakeholders can improve the value of risk assessments for environmental decision making by addressing three interrelated issues: transparency, objectivity, and communication. Stakeholders should be involved in risk assessments throughout the entire process-from assessment planning and problem formulation (including selection of protection goals and assessment endpoints) to risk communication-to help ensure that assessments are meaningful and informative to the environmental decisions that affect them. Acceptance of assessment results by stakeholders and decision makers depends in part on transparency in how a risk assessment is conducted, including its approaches, methods, assumptions, and uncertainties. Although full engagement by stakeholders in all assessment activities might not be possible or practical, assessments will become more transparent as stakeholder involvement increases. Stakeholders can serve as a check on the objectivity of risk assessments, helping to ensure that the methods employed (or in a worst case, special interests) do not bias results unintentionally or otherwise. They should be viewed as important sources of relevant knowledge about specific environmental systems and how people interact with them, and of the values people impute to those systems. Engagement of stakeholders throughout the process facilitates communication of risk assessment results and the rationale and expected benefits of management decisions based on them. Stakeholder engagement promotes clarity and understanding of the approaches taken during, the results obtained by, and the limitations of risk assessments, and should result in wider acceptance of this tool

as valuable for informing environmental decisions.

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Reducing Nonpoint Sources of Nutrients via Non-traditional Approaches
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Communities in the U.S. are seeking innovative and cost-effective approaches to manage nutrient loading from nonpoint sources in ways that improve environmental and societal conditions. This project focuses on providing watershed-based solutions for nonpoint source nutrient loading that can support communities across the United States and beyond. Our primary objective is to test nationally-relevant approaches for managing nonpoint source nutrient pollution in partnership with the Barnstable Clean Water Coalition (BCWC), a non-governmental organization located in the Three Bays watershed on Cape Cod, MA where nonpoint source nutrient loading is a significant problem. Resolving this issue through installation of public sewer systems, while technologically feasible, is extremely expensive due to the distribution, density, and seasonality of the population on Cape Cod. To best address nutrient pollution in an efficient and economic manner requires understanding the waterbody and its associated watershed and designing tailored management controls. The BCWC is promoting nature-based solutions to reduce nutrient loadings that will achieve its water quality goals quickly and cost-effectively. Objectives for BCWC in this partnership include: 1) solving the nutrient problem in Three Bays in a sustainable and affordable manner; 2) becoming a recognized national model for addressing non-point nutrient management; and 3) becoming a center for education and outreach to support other communities as they address issues of nonpoint source nutrient management. This project supports BCWC and other stakeholders in meeting their goals for restoration and water quality in a manner that is transferable to other systems nationwide. This research contributes directly to the monitoring (performance evaluation of interventions and quantification of corresponding watershed-level responses), modeling (best placement of interventions on the landscape, and effect of interventions on estuarine waterbodies), restoration (cranberry bog test systems and freshwater pond restoration), and social science (economic valuation associated with clean water and tourism; acceptance of various interventions; data visualization and science translation) needs of BCWC to achieve comprehensive and watershed-scale solutions to nonpoint source nutrient loading. Feedback and information gathered at the Problem Formulation Workshop will be presented, as well as ongoing stakeholder engagement activities.

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Developing Regulatory guidance that meet Regulatory Objectives and Field Reality: learning from the MAgPIE project
A. Alix, Corteva Agrisciences / Risk Management
The development of regulatory guidance documents that address regulatory needs and protection goals while still reflecting field reality is may be of increase challenge. This reflects at least two aspects of regulatory risk assessment: 1) the increasing scientific knowlegde in the research fields related to a regulatory matter, that results in the increasing awareness of the actual complexity of that scientific area and 2) the increasing involvement and legitimate demand from the general public, for safer chemistries, practices and production systems, which need to be taken into account by decision makers. Yet the resulting complexity in updated regulatory guidance may not be received as a necessary improvement by all stakeholders, particularly in situations where they perceive a disconnection with their own understanding of the issues and their potential solutions. As a consequence valuable knowledge and guidance may simply be ignored, contourned, disregarded with ultimately no benefit for human and environmental protection. Risk mitigation measures are of increasing importance for environmental protection in the area of the use of pesticides in crop protection. In 2012, the question raised multiple exchanges between European authorities, and many initiatives had been undertaken in order to develop, implement and account for risk mitigation measures in the risk assessment procedures. As a result of these exchanges, it was decided to organise a European workshop in order to encourage further the dialog and provide European regulatory authorities a toolbox of risk mitigation measures designed for the use of Plant Protection Products for agricultural purposes. But most importantly, it was felt critical to keep the scientific discussion as open as possible and involve stakeholders to reflect the whole chain of contributors, from risk assessors to end users (farmers). This contribution offers to present the approach followed by the MAgPIE workshop organisers and the process that lead to develop this European risk mitigation toolbox. The MagPIE workshop was organised under the auspices of SETAC and the European Commision.

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Systematic Maps as tools for the translation of research into regulatory decisions
T. Wolffe, Lancaster University; P. Whaley, C. Halsall, Lancaster University / Lancaster Environment Centre
The volume and breadth of accessible data of potential relevance to chemical risk management is continually growing, as our understanding of the mechanisms underpinning toxicity and our appreciation for the behavior of chemicals in the environment continues to develop. Although this has significant implications for informing regulation, the volume, scope and heterogeneity of this data can present significant challenges for risk assessment and risk management decision-making. In the absence of a sufficiently robust and transparent framework with which to collate and handle this data, decisions makers risk cherry picking evidence and allowing observer bias to steer its interpretation, compromising the translation of research into regulatory action. These challenges have seen an increasing interest in systematic mapping as a tool for regulatory decision making in environmental health. Systematic maps provide the framework needed to collect, process and store all evidence available on a broad research topic, such as the effects of exposure to a chemical substance. They distil a vast, heterogeneous evidence base into an accessible, comparable and easily updated format using transparent and reproducible methodology. Their broad overview facilitates fast identification of emerging trends, including the presence of evidence gaps and evidence clusters, which can be used to target resources more efficiently. This talk introduces the concept of systematic mapping to the chemical risk management workflow, and identifies the highly connected nature of environmental health data to be vital for understanding trends in the evidence base. Current data management practices, which struggle to preserve these connections and therefore limit the value of existing data, will be contrasted to the advantages of adopting graph database technology for systematically mapping the risk assessment relevant evidence base. The applicability of graphical systematic maps to predictive toxicology, and their amenability to advances in machine learning and AI, will also be discussed in the context of challenges faced by regulators; i.e. an ever-expanding pool of chemicals requiring evaluation, increasingly pressed resources with which to conduct evaluations, and the potentially uninformative nature of traditional toxicity tests used to conduct these evaluations.

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Tailings spills continue to happen: so why do we seem to always get the response wrong?
R. Smith, A. Markham, Hydrobiology Pty Ltd
On November 5, 2015 the Fundao Tailings Dam at the Samarco Iron Ore Mine failed in a liquefaction flowslide. An estimated 32 Mm^3 of tailings plus scoured valley colluvium and alluvium was discharged downstream, initially as a debris flow, along some 680 km of the Rio Doce in Brazil. This was the largest mine tailings spill to date and one that continues to garner international press coverage. We were called in to assist one of the shareholder companies with technical advice on their response within 48 h, but over 3 years later we are still heavily involved in a contentious multi-sectoral, multi-party assessment of the impact and recovery trajectory of the spill. With over 30 years each dealing with tailings releases into water courses, from small to large, in developing countries and developed countries, we conclude that across our experience, such disharmony is prevalent and basic errors continue to be needlessly repeated in the response to spills. We propose that the mining industry as a whole, has not adopted the strategies of preparedness for disaster of the petroleum industry and continues to respond with shock at the occurrence of systems failures leading to spills, and regulators continue to see penalty of those responsible as the favoured response over facilitation of recovery. We propose a paradigm of preparedness and stakeholder collaboration as a much preferred, logical alternative.

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Can living organisms in bioassays be replaced on enzymes? From idea to commercial products. Problems and advantages.
V. Kratasyuk, Siberian Federal University / Biophysical
Historically, the application of bioluminescence in toxicology began with the usage of luminous bacteria and they are still widely used. As opposed to other test objects such as paramecia, algae, and so on, the luminous bacteria assay is faster (< 30 min). However, as with other organisms, luminous bacteria is petulant. The failure to maintain the stable state of bacterial culture during measurements and storage results in low accuracy of measurement, a clear disadvantage of this method caused by the "petulance". The bacteria react to the toxic substances either by decreasing or by increasing the luminous intensity, often leading to ambiguous interpretation of results. Because of these shortcomings the luminous bacteria assay didn't show reliable results. The new approach to develop the bioluminescent enzymatic biosensors, toxicity bioassays and reagents has been described. To solve the problem of how to detect, identify, and measure the contents of the numerous chemical compounds in environmental monitoring, food product monitoring, and medical diagnostics, the bioluminescent enzymatic toxicity assays were proposed, wherein the NAD(P)H:FMN-oxidoreductase +luciferase substitutes for living organisms. The immobilized reagent Enzymolum was introduced to facilitate and accelerate the development of cost-competitive

enzymatic systems for use in biosensors. Prototype biosensors offer cost advantages, versatility, high sensitivity, rapid response, extended shelf and flexible storage conditions. Due to the coupling with luciferase, it is possible to design new enzymatic bioassays in toxicology and combine them into a set. The set includes key enzymes of metabolic processes such as LDH, trypsin and others. The set of bioluminescent enzymatic toxicity assays was used for monitoring natural and laboratory aquatic ecosystems, soil contamination, as well as for toxicity analysis of pesticides and sanitary assessment of nanomaterials. The new possibilities of enzymatic bioassays are discussed. We are ready to say and discuss the problems which were arising during developing this new approach. At the beginning it was very hard to discuss with our peers use the new perspective as key to our idea. But even now when we win we have problems with understanding of the new approach and wish to show the way and history of developing bioluminescent bioassays from idea to commercial products. The research was supported by the Russian Science Foundation, project No 16-14-10115.

Trans-Disciplinary Research on Coastal Ecosystems of the Northern Europe: Achievements and Problems

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Diversity and typical structure of salt stratified lakes on the White Sea coast

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Many coastal lakes and lagoons at different isolation stages were formed on the White Sea coast as a result of isostatic postglacial uplift. Lakes undergo gradual transformation from a sea bay into a continental water body, thereforerepresent different steps of hydrological evolution and ecological succession. Halfway through their evolution,a strong stratification settles up to the meromixis. To date, we have investigated about 20 coastal semi-isolated lakes and lagoons. Bathymetric studies were carried out, vertical profiles of temperature, salinity, pH, ORP, dissolved oxygen, hydrogen sulfide, and nutrients were obtained. Long-term monitoring is being carried out in several lakes in all year seasons during over 10 years. The sharpness of physicochemical gradients and stability of vertical stratification depend on the interaction of freshwater runoff and the frequency of seawater intrusions. Long-term temperature trends have been identified. According to vertical structure data on quite a number of studied lakes, a generalized hydrological scheme was constructed. On the boundary of aerobic and anaerobic zones, colored water layers appear caused by blooming of some eukaryotic microorganisms, including mixotrophs, or anoxygenic phototrophic bacteria. Colored layers enhance sharpnessof physicochemical gradients and make the stratification more stable. As a result of phytoplankton photosynthesis, a layer with extremely high concentration of dissolved oxygen (up to 300% saturation) often emerges in the aerobic zone. The adjoined bottom zone is characterized by a particularly high abundance and biomass of macrozoobenthos. In the lagoons with the regular tides *Macoma balthica* community prevails; while reservoir's isolation increases, it is replaced by *Hydrobia ulvae-Chironomus gr. salinarius* association; in water bodies with freshwater mixolimnion, insect larvae, mainly chironomids, are dominant. Some lagoons have the thickness of all typical water layers enough to form a bottom zone with specific macrozoobenthos associations. The surface water layer with a lower salinity is characterized by the greatest species diversity due to insect larvae varying from year to year. Marine fauna typical for neighboring shallow waters of the White Sea inhabits the bottom depth which contacts to the saline aerobic layer, and the anaerobic zone lacks of macrobenthos.

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Copper toxic effects on sea star Asterias rubens at different levels of biological organization

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The common sea star *Asterias* rubens represents a key-species of the White Sea macrobenthic community. Copper is abundant anthropogenic source metal and one of most toxic in marine sediments. The toxic effects of various concentrations of copper on survival, the behavioral response, changes in cellular elements of coelomic fluid (CF) of starfish *Asterias* rubens and heat shock protein 70 (HSC70) concentration and coelomic cells viability in response to exposure were investigated. Half-lethal concentrations were established for copper (0.98±0.16 μM). Exposure of this metal led to a significant righting time growth. When the sea stars were exposed to Cu it accumulated in the bodies and the number of circulating coelomocytes, increased significantly. Copper exposure resulted in increase agranulocytes and small cells proportion on other side decreasing in the percentage of red amoebocytes (granulocytes) in the CF. In addition, coelomocytes showed stress response in terms of changes in HSP70 levels and as judged by Western blotting. Taken all together, our results confirm the potential of sea stars *Asterias* rubens as bioindicator and their coelomocytes as biomarkers of environmental stress with different sensitivity at different levels of biological organization.

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Adaptive potential diagnostics in selected marine invertebrates for assessing ecological status (health) of coastal marine ecosystems

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The aim of the present paper was to review the results obtained in our previous field and laboratory studies using cardiac monitoring system worked out at Saint-Petersburg Scientific Research Center for Ecological Safety for assessing of biological effects of environmental pollution in a few problem costal seas. The experience of development and approbation of proposed approach to the assessment of biological effects of environmental chemical stress based on evaluation of adaptive potential of indigenous species of the invertebrates from different in anthropogenic pressure fresh water, brackish water or marine areas, with the emphasize on different sea is discussed. The evaluation of adaptive potential of representatives of local biota (mollusks and crustacean) was based on measuring the heart rate recovery time after removal of stress load (50% salinity change). Rapid recovery (less than 50-60 min) signifies a good adaptive potential in different species, indicating good ecological status of the study site they inhabit. The paper presents a number of examples demonstrating how methodology for the evaluation of invertebrate's physiological state can be used in ecosystem health assessment and ecological risk assessment in the Baltic Sea Region, the White Sea,the Black Sea (Crimea) and the Adriatic Sea (the Boka Kotorska Bay). The later approach has been successfully applied in laboratory and mesocosm experiments to assess the toxic effects of hazardous substances on marine organism status and could be a useful tool in screening of biological effects of emerging agents with the unknown mode of action. "The study is supported by the Grant 18-44-920010 "Assessment of the recreational potential of the waters of the Sevastopol region by bioindication methods"\n

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Fluorescence and absorption spectroscopy of chromophoric dissolved organic matter (CDOM) in aquatic environments of the Northern part of European Russia

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The colored/chromophoric dissolved organic matter (?DOM) is present in all types of natural water. Its typical concentration in water is low, however the ?DOM is a significant organic carbon reservoir on the Earth, exceeding the organic matter of living organisms. Since the ?DOM of natural origin absorbs the UV and short-wave visible light and therefore emits fluorescence, its optical spectra are successfully used in the tasks of monitoring the natural aquatic ecosystems. For the aquatic CDOM in various types of natural aquatic environments (marine, riverine, stratified relic lakes) such spectral characteristics as the wavelength of the emission maximum in fluorescence spectrum, the fluorescence quantum yield, the spectral dependences of optical density and their higher derivatives are very significant. Spectroscopic measurements were performed to analyze water samples from coastal zones of the White Sea, Baltic Sea, several river and lakes, freshwater and saline, located in Karelia to characterize CDOM origin and to reveal its fluorescent components. Fluorescence quantum yield and emission maximum wavelength were described as a function of excitation wavelength within wide spectral range from 230 to 560 nm. The "blue shift" of fluorescence emission was observed for all the CDOM samples with change in excitation wavelength from 270 to 310 nm. The emission maximum suffered the 'red shift" at excitation wavelengths above 310-340 nm. Fluorescence quantum yield reached maximum at excitation wavelengths about 370 nm and decreased monotonically thereafter. The similarity of spectral characteristics in different freshwater lakes with that observed for coastal and terrestrial CDOM samples revealed that CDOM in coastal zones is composed of a major terrestrial component. Such technique can be successfully used in aquatic ecosystems monitoring. This work was supported by the Russian Foundation for Basic Research (RFBF) under Grant Number 18-016-00078 and by a grant from the "Bazis" Foundation for Development of Theoretical Physics and Mathematics.

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Unlike relatives and unrelated doubles in the world of microbes by the example of anoxygenic phototrophic bacteria from the lakes connected to Kandalaksha Bay, White Sea

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The phylogenetic position of anoxygenic phototrophic bacteria from the chemocline zone in stratified lakes connected to the Kandalaksha Bay was investigated. In lake Kislo-Sladkoe we discovered the 100% phylogenetic similarity (p/g sim) of 3 isolatedgreen sulfur bacteria (GSB) morphotypes. One brown-colored (b/c) *Phv*PS10 (GenBank (GB) ??702853) strain with bacteriochlorophyll (BChl) *e* and carotenoid isorenieratene. Two green colored (g/c) (*Chlv*PS10 (GB ??702852) and *Pr*PS10 (GB ??702854)) strains with Bhl *d* and carotenoid chlorobactin. All strains had p/g sim with *Chlorobium phaeovibrioides*: b/c DSM 269^T (Y08105) – 99.3%, and g/c DSM 265 (GB NC_009337) – 100%. Two the same identical strains b/c *Phv*Tcv-s14 (GB MH509941) and g/c*Gr*Tcv13 (GB MH509940) were also found in lake Trekhtsvetnoe. From lagoon on the cape Zeleny a b/c GSB strain ZM (GB PDNX01000007.1) with prosthecas was isolated, which contained Bchl *e* and isoreniaratin. It had almost 100% p/g sim with the g/c *Prosthecochloris aestuarii* DSM 271^T(GB Y07837). Purple sulfur bacteria (PSB) *?cr*PS10 (GB KC702858) and *?m*PS10 (GB KC702856) strains from lake Kislo-Sladkoe were similar and phylogenetically identical, contained carotenoid okenone, could be motile due to a bunch of flagella or nonmotile with gas vacuoles and most resembled the okenone-contained bacteria of *Lamprobacter* and *Lamprocystis* genera. However, p/g sim with ? *Lpb. modestohalophilus* Sivash^{NT} (GB HQ877095) was 93%, with *Lpc. purpurea* (GB NR_119250.1) – 90%. The closest strains were *Thiocapsa*: *Tca. rosea* DSM 235^T (GB AJ006062) spirloxanthin-contained with gas vacuoles – 99.1%, okenone-contained without gas vacuoles *Tca. marina* (GB Y12301) – 99%. Saltwater PSB *Trcc*PS10 strain from lake Kislo-Sladkoe looked like *Thiorhodococcus drewsii.* The main carotenoid was rhodopin, bacterial suspension had a reddish-brown color. However, the strain occupies a separate intermediate position between pink-purple rhodopinal-contained type strain *Trc. kakinadensis* DSM 18858^T (GB AM282561, 98.3% p/g sim) and rhodopin-contained strain *Trc. drewsii* DSM 15006 (GB FM178273, 97.5% p/g sim). Thus, the type of Bhl and carotenoids in GSB, and even the presence or absence of flagella or gas vacuoles in some PSB, do not determine the species of anoxygenic phototrophic bacteria. The work was supported by the RFBR 17-04-01263. The work was carried out using the scientific equipment of Core Research Facility “Bioengineering”.

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Chlorophyll fluorescence methods in biomonitoring of water bodies separated from the White Sea
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Phytoplankton are the primary trophic level of a food chain in aquatic ecosystems. Their functional state, especially photosynthesis, can be affected by ecological factors leading to changes in the entire food web. According to this fact, monitoring of physiological state of phytoplankton assumes ever-greater importance. Its successful implementation should provide an opportunity to detect alterations of the individual levels of ecological systems such as aquatic phototrophs and, based on this, to predict further changes of entire aqueous systems over the time. Particular importance is obtaining a relevant information of cellular metabolism alterations of photosynthetic organisms before morphological symptoms become visible or even before changes in chlorophyll content become apparent. Nowadays, luminescence methods, in particular fluorescence, are being developed to detect alterations of the physiological state of photosynthetic organisms. Approaches based on registration of chlorophyll fluorescence are rapid, non-invasive and non-destructive methods that allow recording an immediate response of photosynthetic organisms to the changes of environmental factors *in situ* due to chlorophyll in the photosynthetic membranes serves as a natural probe of the photosynthetic organisms. There are different principles of registration of chlorophyll fluorescence that permit to investigate the photosynthetic processes at various functional levels (e.g., light-harvesting complexes, primary light reactions, electron transport chain, light-independent reactions in thylakoid stroma, and even slow regulatory processes). For the first time, chlorophyll fluorescence methods were applied in biomonitoring of water bodies separated from the White Sea. Application of chlorophyll fluorescence methods allowed to characterize physiological state of photosynthetic organisms that inhabit separated water bodies, and to reveal features of their photosynthetic behavior.

Linking Models, Experiments and Measurements to Reliably Investigate the Environmental Fate and Effects of Hydrophobic Organic Contaminants and Mixtures (II)

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Passive dosing and aquatic toxicity testing of complex organic mixtures - Linking toxicity to concentrations in the silicone and lipid donors
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Environmental hazard and risk assessment of complex organic mixtures is complicated because the individual constituents in the mixture have various physicochemical properties and toxicity. One important experimental challenge is to establish and maintain well-defined exposure concentrations to such mixtures in an aquatic toxicity test. An improved experimental methodology can subsequently lead to a better toxicity assessment. The present study, therefore, aims to (1) enable aquatic toxicity testing of complex mixtures at defined and controlled exposure and (2) provide approaches to assess whether the test mixtures exhibit baseline or excess toxicity based on their concentrations in the silicone or lipid donor. Two passive dosing (PD) methods were applied to dose-response testing of complex mixtures with the fresh water crustacean *Daphnia magna*. The first method used a polydimethylsiloxane (PDMS) silicone rod as donor (i.e. silicone PD), which was placed in the aqueous solution and headspace, whereas the second method used a lipid donor (Miglyol oil) for the passive dosing only via the headspace (i.e., headspace PD, HS-PD). The test mixtures were several petroleum products and essential oils. These mixtures were loaded to PDMS rods and Miglyol oil by weighing to desired mass concentrations (%) in these donors. The exposures to test mixture at the saturation level in the two PD systems were analytically confirmed against the pure test mixtures using headspace GC-MS and it was also shown that these two PD methods cross validated each other. The EC_{50} values for the petroleum mixtures and essential oils in the *D. magna* acute immobilization tests ranged between 5-6 % and 0.6-3.2 % mass concentration, respectively, on the silicone base. The EC_{50} values on a lipid base ranged between 16-32 % for petroleum mixtures and 8.5-39 % for essential oils. The EC_{50} values for all tested mixtures on the lipid base ranged between 85-390 g mixture per kg lipid, which corresponds to 425-1950 mmol kg^{-1} lipid when assuming an average molecular mass of 200 g mol^{-1} for the tested mixtures. These lipid based EC_{50} values were above the reported concentration range of 40-160 mmol kg^{-1} lipid, which thus indicates baseline rather than excess toxicity. The present study provided an improved experimental basis for aquatic toxicity testing and assessment of complex organic mixtures.

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Thermodynamic assessment of (semi-)volatile hydrophobic organic chemicals in WWTP sludge - combining Solid Phase Microextraction with non-target GC/MS
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Thousands of organic chemicals from households and industry end up in municipal wastewater treatment plants (WWTPs) of which a certain fraction will end up in the sludge[1]. The preferred recycling option in the EU is application on arable soils[2]. However, the sludge can accumulate chemicals and transfer them when applied to the environment and there is thus a need for better understanding the behaviour and fate of hydrophobic organic chemicals (HOCs) during WWTP processes and subsequent treatments of the sludge prior land application. The fate and exposure of a HOC in sludge are closely linked to its chemical activity, which is directly related to its chemical potential[3], and can facilitate comparisons across matrices with different sorbent properties and capacities[4]. Chemical activity can be measured with equilibrium sampling devices that utilize the partitioning of freely dissolved molecules between a matrix and a polymer reference phase until reaching a thermodynamic equilibrium (i.e., the same chemical activity in polymer and sample)[5]. Solid Phase Microextraction (SPME) with a polydimethylsiloxane coated fibre is such a partitioning based method that can be used for measuring chemical activity[6]. SPME can be fully automated, having the advantage of both high throughput and high reproducibility. The method was developed and applied to compare chemical potential of (semi-)volatile HOCs in sludge stages across a WWTP and to determine the reduction in chemical potential (*i.e.* exposure level) of (semi)volatile HOCs during co-composting and sorbent amendment of digested sludge. Generally, chemical activity increased from primary, to secondary, to digested sludge and the level in dewatered sludge was not significantly different from the level in the digested sludge. Co-composting end-treatment was generally effective in reducing the chemical potential of (semi-)volatile HOCs. References:
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Effect of lipid digestion on PCBs transfer from microplastics during in vitro digestion

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Human intake of microplastics through ingestion is not surprising especially since microplastics have been detected in several components of the human diet, including drinking water. It is unknown to what extent exposure of these microplastics would lead to negative effects. While there is some knowledge on particle toxicity effects using in vitro cell line cultures for nanoparticles, and until more recently for microplastics, the potential risks of plastic-associated chemicals as the microplastics pass through the gastrointestinal tract is still uncertain. These particles act as vectors for chemicals (i.e. sorbed contaminants and additives) and the bioavailability of these chemicals during the gut retention time of humans is uncertain. To date, risk assessments of associated chemicals have assumed full release of chemicals from microplastics when ingested. However, the bioavailability of these chemicals depend on chemical transfer kinetics, fugacity gradients and retention times of the particles. To provide a better assessment of the human health risks for plastic-associated chemicals, the mechanisms of chemical transfer from microplastics, as well as the other way around, first needs to be elucidated. We developed an in vitro system, which simulates the small intestinal conditions, to study the chemical exchange kinetics from microplastics. As lipids and plastics are known to have high affinities for hydrophobic organic contaminants (HOCs), a range of PCBs with different hydrophobicities were used as model HOCs to understand the effect of lipid digestion on chemical exchange in microplastics. We compared the differences in the kinetics between two most frequently used polymer types for food packaging, i.e. low density polyethylene (LDPE) and polyethylene terephthalate (PET). Knowledge of these kinetic rates allows us to better understand the proportion of chemicals bioavailable and absorbed in the gastrointestinal tract. After exposure of 3-4 h, about 10-68 % of chemicals may potentially leach out and be bioavailable for absorption based on the scenario of the experiment. As chemical bioavailability also depends on the relative concentrations between the gut fluids and plastic, a systematic evaluation of different scenarios using the derived kinetic parameters from this study would provide insights into the potential risks of plastic-associated chemicals.

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PUF Disk Samplers for POPs, Metals and PM in Air: Spatial Scales, Human Health, and Future Research

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Polyurethane foam (PUF) disk passive air samplers have been widely applied for more than a decade to study semivolatile organic chemicals in air. The samplers are simple to use, cost-effective and their electricty-free operation allows them to be deployed anywhere. The passive sampling approach trades-off temporally resolved data, possible with high volume active air samplers, for spatially resolved data that is relatively easily to obtain over local, regional and even global spatial scales – for instance the Global Atmospheric Passive Sampling (GAPS) network and the Global Monitoring Plan (GMP) of the Stockholm Convention on POPs. In many instances, the spatially resolved data is a more relevant and pragmatic approach for investigating abundances in air, long-range transport behaviours, effectiveness of chemicals management measures (temporal trends), and for validating models. Very recently, the use of the PUF-disk samplers has been extended to investigations of particulate matter and metals in air and linking contaminant burdens with indicators of toxicity for the mixture of chemicals in air (e.g. mutagenicity, cytotoxicity, oxidative potential) by using in vitro and chemical assays. Results are presented from local, regional and global-scale programs employing the PUF disk sampler. These include the PAC-MAN (Polycyclic Aromatic Compounds- Monitoring Air Network) in the oil sands region of Canada, ATOUSSA (Assessing Toxicity of Urban Source Sectors for Air) study in Toronto, and a new GAPS Megacities project that investigates POPs, metals and toxicity across 19 international megacities. Future applications and cross-linkages of the PUF disk sampler are considered including: i.) assessing black carbon, a short-lived climate forcer, in air; and ii.) investigating microplastic burdens and transport in air. Furthermore, a global PUF disk sample bank is being established, covering the period 2010 to present. This PUF disk archive presents an opportunity to investigate a wide range of current and priority contaminant issues over space and time and to inform global emissions and model development.

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Time trend comparison of co-located active (EMEP) and passive (MONET) sampling sites monitoring persistent organic pollutants in European air

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Two transnational persistent organic pollutant (POP) air monitoring networks currently operate within Europe: EMEP active (ACT) sampling (NILU) and MONET passive (PAS) sampling (RECETOX). While ACT sampling of POPs in air has been ongoing at several EMEP sites since the 1990s, MONET PAS samplers were deployed in the 2000s as an alternative method to address gaps in monitoring due to their ease of use and lower cost. Unfortunately, the comparability of ACT and PAS sampling data is confounded by uncertainty in the influence of sampling rates and particle-phase dynamics on the recalculation of air concentrations; differences in the duration, volume and frequency of sampling between ACT and PAS networks may also hinder comparability. To address these challenges, we calculated and compared time trends for organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) at the six sites with at least 5 yr of co-located ACT and PAS sampling data (2012–2016): Birkenes (Norway), Kosetice (Czech Republic), Pallas (Finland), Rao (Sweden), Storhofdi (Iceland), and Zeppelin (Norway). ACT air data were obtained from EMEP as monthly aggregate means (ng/m^3). MONET PAS samples are analysed at RECETOX and reported as ng/PUF/sampling period (~84 d). Time trends and halving-times for each site and compound were calculated using the Theil-Sen estimator method. Agreement of corresponding ACT and PAS trends was then estimated using the Mann-Whitney U test. ACT trends for OCPs and PCBs showed strong and significant negative correlations, although certain site and compound exceptions occurred. PAS trends were similar in most cases, albeit with lower strength and significance due to the shorter length of monitoring and less frequent sampling. Agreement of ACT and PAS trends varied by site and compound class with PCBs and HCHs exhibiting the strongest comparability while HCB and pp-DDE were the most prone to disagreement. Since PAS trends were calculated using significantly fewer data (12 ACT samples/yr for 15+ yr vs. 4 PAS samples/yr for 5+ yr), comparability to ACT trends may be improved with additional years of data. Strong agreement between trends for most sites and compounds suggests PAS sampling can be used for long-term air monitoring of POPs where ACT sampling is impractical or unfeasible. However, differences in sampling rates and limitations on recalculation still hinder the direct comparison of individual POP concentrations from ACT and PAS air monitoring networks.

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How much do dietary and non-dietary sources contribute to prenatal exposure to BPA and DEHP? A Catalonia (Spain) case study.

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Endocrine disruptors (EDs) are chemicals compounds that send confusing messages causing various dysfunctions by mimicking or altering the effect of hormones in the human body. Several EDs such as Bisphenol A (BPA) and di (2-ethylhexyl) phthalate (DEHP), are involved in obesity and diabetes diseases in children. Recent studies have shown evidences that these chemicals can cross the placental barrier making fetal exposure closely related to maternal exposure. The aim of this research is to establish fetus exposure to EDs (BPA and DEHP). To address this issue, recently, pregnant women recruitment has begun. In present work, dietary and non-dietary (dermal, non-dietary ingestion and inhalation) exposure of these women was considered. To estimate prenatal exposure, a physiologically based pharmacokinetic (PBPK) model was adapted for pregnancy. Estimates of exposure from all pathways along with their relative importance were provided in order to establish which proportion of the total exposure came from diet and which from non-diet. Total non-dietary mean values were 0.002 $\mu g/kg_{bw}$/day (0.000; 0.004 $\mu g/kg_{bw}$/day for 5th and 95th percentile, respectively) for BPA and 0.597 $\mu g/kg_{bw}$/day (0.116 $\mu g/kg_{bw}$/day and 1.506 $\mu g/kg_{bw}$/day for 5th and 95th percentile, respectively) for DEHP. Indoor environments and especially dust ingestion were the main non-dietary contributors to the total exposure of BPA and DEHP with 60% and 81%. However, as expected, diet showed the higher contribution to total exposure with >99.9% for BPA and 63% for DEHP. Although diet was considered the primary source of exposure to BPA and phthalates, non-dietary sources need to be more thoroughly characterized because with these sources the first-pass metabolism is lacking, so these may be of equal or even higher toxicological relevance than dietary sources. This work is included in the frame of HEALS project (FP7-603946).

Poster Abstracts

Remediation of Contaminated Environments and Innovative Methods in Biological Strategies (P)

MO001

Can the anionic surfactant sodium lauryl ether sulphate (SLES) affect the persistence of the pesticide chlorpyrifos in soil?

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Chlorpyrifos (CPF) is an organophosphate insecticide intensively used in agricultural practices. It is persistent in soils, from which it can reach the aquatic environment. Natural biodegradation can play an important role in its removal from the environment. Surfactants are among the substances that can improve the bioavailability of pesticides for natural microorganisms.They are amphipathic molecules able to solubilize hydrophobic contaminants as pesticides through their incorporation into the hydrophobic core of micelles in solution.Sodium lauryl ether sulphate (SLES) is an anionic surfactant widely used in the underground excavation industry, as it is the main component of foaming agents exploited as lubricants. SLES residual concentrations can occur in the spoil materials reusable as by-products for different environmental purposes, such as mixing to agricultural lands. To date, no studies have been carried out on the possible interactions between this surfactant and pesticides in soils. The aim of this study is the evaluation of the persistence of CPF in an agricultural soil in the presence/absence of SLES. Both the CPF and SLES half-life (DT_{50}) were evaluated and the possible effects on the soil natural microbial community were assessed. For this purpose, several replicated microcosms containing uncontaminated soil (Ctr), soil with only SLES (70 mg/kg) or only CPF addition (2 mg/kg) and soil with both SLES and CPF addition were set-up. At selected times (0,7,14,21, 28 days), soil sub-samples were collected from microcosms to determine their residual concentrations. Analysis of SLES was performed with Methylene Blue Active Substances (MBAS) method, while CPF was analysed with GC-ECD, both preceded by Pressurised Liquid Extraction (PLE) from soil. Microbiological analyses were performed in order to assess microbial abundance (DAPI counts), cell vitality (live/dead method), dehydrogenase activity and structure (ELFA analysis). The results showed that both CPF and SLES concentrations decreased during the experimental time. CPF halving time was not significantly affected by the presence of SLES (DT_{50}10 d when alone *vs*11 d when in the co-presence). Even if, the results highlighted that in the co-presence of SLES, all the microbial populations were favoured in term of abundance and activity because they were able to degrade both the pesticide and the surfactant, showing more versatile metabolic capacities.

MO002

Microbial Fuel Cells (MFCs) for promoting DDE degradation

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DDT was one of the most used synthetic pesticide worldwide. It was used in a great variety of applications until 70's when it was forbidden in most countries because of its toxicity and its deleterious impact on the environment. DDE (1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene) is one of the main metabolites of DDT which is produced through biotic degradation in aerobic conditions, abiotic dehydrochlorination and photochemical degradation. It is a polychlorinated compound with a high hydrophobicity, high persistence, high potential of bioaccumulation through the trophic chain. Although the use of DDT has been prohibited for decades, residual DDE concentrations are ubiquitous in the environment. Recently, several bioremediation technologies (e.g. using plants and fungi) have been developed to remove DDT and DDE. Among green technology, the use of Microbial Fuel Cells (MFCs) is a promising one for stimulating pollutant bioremediation based on the conversion of the energy stored in the chemical bonds of organic compounds into electrical energy. This study is focused on the effectiveness of MFCs in improving degradation of DDE in soils stimulating exo-electrogen microorganisms that catalyze oxidation and reduction reactions at two electrodes (anode and cathode). Bacterial populations tend to develop making a biofilm producing electrons and protons at the anode, which it used as an electron acceptor under anaerobic conditions. Electrons flows from the anode to the cathode through an external circuit that connects the two electrodes and thus create current. Protons flow through the soil to the cathode where, in aerobic conditions, electrons and protons react with oxygen to produce water. In this study, 4 experimental conditions using MFCs (open circuit) have been carried out (3 replicates each) for evaluating DDE biodegradation: soil, soil + compost, soil + DDE, soil + compost + DDE. Moreover the last 2 conditions were tested both using opened and closed circuit through different electrical resistances to obtain the maximum power value corresponding to the highest DDE degradation rate. Control soils (without MFCs) were used as controls. Chemical and microbiological (microbial abundance, cell viability, DHA) analyses and electrical measurements have been performed at three sampling times: 0 day, 2 months and 4 months.

MO003

IDENTIFICATION OF BACTERIA INVOLVED IN SODIUM LAURYL ETHER SULPHATE BIODEGRADATION IN CONDITIONED SOIL FROM A TUNNELLING CONSTRUCTION SITE

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The anionic surfactant Sodium Lauryl Ether Sulphate (SLES) is the principal component of several commercial foaming products for soil conditioning in the tunnelling industry. Huge amounts of soil debris are produced during the excavation process and the presence of SLES can affect the re-use of the spoil material as a by-product. SLES biodegradability is a key point for the re-use of the excavated soil. Recent studies showed that SLES degradation depends on the presence of natural microbial populations colonizing the spoil material in the temporary deposit in the construction site. However, the degradation rates are affected by site-specific conditions consequently SLES persistence can vary under different environmental conditions.The aim of this study was to identify SLES degrading bacteria and to explore their bioremediation capacity. Enrichment cultures were performed using as inoculum bacterial populations from spoil material collected in a tunneling construction site. Approximately 0.5 g of soil (wet weight) was added to 50 mL of minimal medium (MB) enriched with different concentrations of SLES (25, 50, 100, 200 mg/L). All replicates and treatments were treated separately for isolation purposes. SLES degraders were grown in the dark on a shaker at 130 rpm for 7 days at 28°C. Subsequently, 500 mL of the pre-grown cultures from each condition were transferred to fresh SLES enriched media (50 mL) for another 7 days. Appropriate un-inoculated sterile controls were also maintained throughout the experiment. Both SLES concentration and microbial growth were measured. After seven days the highest optical density was observed in the culture medium enriched with 200 mg/L of SLES, therefore it was decided to use this concentration for the isolation experiment. 99% of SLES was biodegraded in just 24 hours. FISH analysis showed that microbial cells involved in the biodegradation belonged to Gamma-Proteobacteria. DNA was extracted and the sequencing of the isolated strain is in progress. The possibility to isolate a natural bacterial strain able to degrade SLES has great potential to be used for bioaugmentation purpose in order to shorten the residence time of spoil materials in the construction sites.

MO004

Bioavailability and cometabolism of pyrene in chemotactic bacteria

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Polycyclic aromatic hydrocarbons (PAHs) are poorly available in soils due to their interaction with the soil matrix. Bacterial chemotaxis promotes the contact probability between degrader organisms and pollutants, increasing bioavailability and thus biodegradation rates.*Pseudomonas putida* G7 is able to use naphthalene as growth substrate, and responds chemotactically to naphthalene and salicylate, a common intermediate in PAH metabolism,showing smooth movement and positive taxis. We used strain G7 as a model organism to evaluate the potential role of motility in the bioavailability of adsorbed high molecular weight PAHs. We conducted batch incubations of strain G7 in mineral medium with ^{14}C-labelled pyrene loaded on silicone o-rings, which acted as a passive dosing system.
The time course evolution of the ^{14}C-pyrene equivalents in culture fluids revealed a linear increase in the presence of G7, achieving concentrations well above the equilibrium concentration observed in abiotic controls. This phenomenon could only be explained by an active biological process. HPLC quantification revealed that in the presence of strain G7 the final concentration of pyrene in the water phase did not change in the presence of salicylate, but it was well below that observed in abiotic controls, indicating the transformation of pyrene into water-soluble metabolites.Interestingly, strain G7 was unable to mineralize pyrene (< 0.1%), indicating that this was a cometabolic reaction. To evaluate the adsorption

of pyrene to cell surfaces, culture fluids were centrifuged and supernatants and bacterial cells were analyzed independently. Only after 48 h a major fraction of ^{14}C-label was detected in the pellet, indicating the sorption of pyrene to bacterial cells. In sand-filled percolated columns we tested how salicylate influenced bacterial transport through a change in motile behavior and promoted accessibility of strain G7 to distantly located ^{14}C-pyrene, dosed in o-rings. Compared to chemoeffector-free controls, salicylate significantly stimulated bacterial transport. The concentration of pyrene in column effluents evidenced that the bacteria were mobilized to the distant pyrene source. Our results indicate that bacterial motility and chemotaxis could be relevant for long-distance bacterial transport in soils, and contribute to cometabolize poorly bioavailable contaminants. This may have implications for the development of more efficient bioremediation strategies.

MO005

Combined effects of biosurfactants and sunflower rhizosphere on the bioavailability of slowly desorbing PAHs in contaminated soil

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Polycyclic aromatic hydrocarbons (PAHs) are the best representatives of chemicals for which specific limitations in bioremediation exist due to low bioavailability. With the objective of enhancing solubilization and consequently decreasing the fraction of bioavailable pollutants, we integrated in a greenhouse experiment different agents to improve bioavailability: microorganisms, plants (sunflowers) and biosurfactants (rhamnolipids R90) in a PAHs contaminated soil with a total concentration of PAHs of 500 mg/kg. These agents can be used sequentially to act on bioavailability, following low-risk bioremediation approaches to treat slowly desorbing chemicals (Environ. Technol. 21, 1099-1110, 2000; Soil Biology and Biochemistry, 57, 830-840, 2013; Environ. Sci. & Technol. 45, 3019-3026, 2011). To measure bioavailability of PAHs we used desorption extraction with Tenax during 20 h (ISO 16751). The contamination level had not significant effects on the plants either seed germination, plant development or flowering. In consequence, sunflower plants tolerated these conditions, confirming their ability to grow in contaminated soils. Measurement of total PAH concentrations showed a decrease by 90 % after 75 days in planted and control treatments. The bioavailable PAHs concentration also declined during this period, indicating that biodegradation efficiently removed most of the fast desorbing chemicals. However, a slight increase in bioavailability was observed in planted soils at day 75, possibly due to plant root decay. Rhamnolipid was therefore added at this point to enhance the biodegradation of the slowly desorbing fractions, once the sunflower plants completed their growth period in the assayed greenhouse conditions. No significant effects were observed by this addition on the decline of total PAH concentrations, however, a significant increase in the rapidly desorbable fraction was observed after rhamnolipid addition in planted soils, what did not occur without plants. Therefore, the biosurfactant made potentially bioavailable a significant fraction of the slowly desorbing PAHs, only when the soil had supported the growth of plants, which possibly released root components or exudates that promoted the biosurfactant action. The positive effect of sunflower roots on soil microorganisms was strongly enhanced when rhamnolipids were added, as a significant increase of dehydrogenase activity in the soil was detected.

MO006

Role of motility behavior in the dispersal of pollutant-degrading bacteria through restricted porosities

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The controlled dispersal of motile bacteria through contaminated soils is currently considered as one of the most promising aspects in bioremediation.The difficulty caused by the heterogeneous distribution of the pollutants and the degrading bacteria can be overcome by the use of different chemoefectors. These compounds allow the control over bacterial tactic motility processes, what results in an increase in the efficiency of the microorganisms dispersion and subsequently, enhanced biodegradation rates (*Environ. Sci. Technol*. 42: 1131-1137, 2008; *Environ. Sci. Technol*. 46: 6790-6797, 2012). A bioreactor system was used, where pollutant bioavailability can be evaluated by means of two chambers separated by a membrane with specific pore sizes. This system allows the study of the role of bacterial plasticity on the penetration through pores that are even smaller than the cell size (*Environ. Sci. Technol. Lett.* 3: 399-403, 2016). The purpose of the present study was to evaluate the behavior of chemotactic bacteria (*Pseudomona putida* G7; cell dimensions: 1 μm x 3 μm) in this system, using different membrane pore sizes (from 0.4 to 12 μm), and determining their dispersal and biodegradation capacity through mineralization tests with the ^{14}C-labelled contaminants. Results are presented here on the first part of the study, focused on bacterial dispersal. This innovative system enabled the control of the experimental conditions to establish specific size ranges to which the bacterium could access the contaminant through a modified motility behavior, including hypermotility (glucose) and chemoattraction (naphthalene and salicylate). The most relevant result in this study was that *P. putida* G7 responded positively to the chemoattractant provided through the membrane pores, crossing the membrane in many cases at higher rates than the control. Penetration depended, however, on cell density and chemoeffector concentrations, and subsequently, on the location of the gradients across the membrane. Progress in this field will undoubtedly open up new possibilities for bioremediation processes in contaminated soils, improving the existing techniques for the treatment of poorly bioaccessible contaminants.

MO007

Rhizoremediation halflives of PCDDs and PCDFs obtained in a greenhouse experiment with seven plant species

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In the last two decades there has been a growing interest in bioremediation technologies which use plants and microorganisms to enhance persistent organic pollutant degradation such as PCB, PCDD, PCDF in contaminated sites (rhizoremediation). Different studies have been conducted to investigate the potential of plant-microbe interactions in the remediation of organic chemical contaminated soils with respect to natural attenuation, providing useful data such degradation halflives. Such a type of data can be used to predict soil concentration temporal trends and the time needed to achieve threshold limits when using plants and their associated rhizosphere microbe to remediate contaminated sites. Although many studies are available for PCBs, few works focus on PCDD and PCDF. In the present work a greenhouse experiment was performed with an aged contaminated soil coming from a National Relevance Site (SIN) located in Northern Italy (SIN-Brescia Caffaro). The soil was characterized by higher concentrations of PCDFs compared to PCDDs, due to higher furan impurities produced during PCB production of the Caffaro Factory. 10 different treatments (combination of 7 plant species and different soil conditions) were considered together with the appropriate controls (soil without plants). Soil and plant biomass were analysed at the beginning of the experiment (t0) up to 18 months (t18). The obtained soil concentrations were used to calculate concentration reduction with time and the corresponding rhizoremediation halflives. The halflives obtained for the most efficient treatments were compared to natural attenuation halflives to evaluate the goodness of rhizoremediation. This study contributed to establish a rhizoremediation half-life dataset for PCDD/Fs

MO008

Bioremediation of diesel-oil contaminated soil with less labor, energy and material by foam and holes

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Petroleum hydrocarbons present in unsaturated deeper soil are especially challenging for the remediation because of uneven delivery and poor contact of reagents with contaminants. The main objective of this study was to enhance the total petroleum hydrocarbon (TPH) removal efficiency within 30-cm deep soil by improving delivery of remedial agents. A column experiment for enhanced bioremediation of 30-cm unsaturated diesel contaminated soil was performed by combining chemical oxidation foam with bioaugmentation foam by means of delivery through the holes. In landfarming microcosm test, when 5% H_2O_2 and 50 mN $Na_2S_2O_8$ solution (0.2 PV of both) were used for 5-cm TPH contaminated soil in tank (24 cm × 17 cm × 16 cm), TPH oxidation efficiencies were 32 and 33% respectively, whereas the removal efficiencies were increased to 80 and 86% after biodegradation using bioaugmentation solution mixing. For the column tests, three columns (25 cm diameter, 70 cm length) were used having 30-cm thick soil and three vertical holes were installed at triangular points. After 0.2 PV of H_2O_2 and $Na_2S_2O_8$ foam spraying pretreatment, the vertical TPH oxidation efficiencies of the soil were in the range of 49.3-53.1%, 47.4-52.8% and 41.2-43.8% from 5, 15 and 25 cm respectively. Two days after oxidation foam spraying, the periodic bioaugmentation foams were sprayed every second day for four weeks (total 1 PV solution). After one month, the concentration of TPH in the soil decreased to 628 mg/kg (90.6% removal efficiency) from 5 cm depth, while 87.8 and 87.6% of TPH were removed from 15 and 25 cm respectively. This study showed that oxidation-bioaugmentation foam spraying using vertical holes improved the distribution of remedial agents throughout the soil with similar pH, higher water content and microbial population as compared to control test. The kinetic study also revealed that a similar and elevated TPH biodegradation rates can be obtained from all soil depths by using this method. (This work was supported by the

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MO009
Microbial diversity and activity of an aged soil contaminated by polycyclic aromatic hydrocarbons
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In-depth understanding of indigenousmicrobial assemblages resulted from aged contamination by polycyclic aromatic hydrocarbons (PAHs) is of vital importance in successful in situ bioremediation treatments. Soil samples ofthree boreholes were collected at 12 different vertical depths. Overall, the dominating three-ring PAHs (76.2%) were closely related to distribution patterns of soil dehydrogenase activity (DHA), microbial cell numbers and Shannon biodiversity index (H') of indigenous microorganisms. High-molecular-weight PAHstends to yield more diverse communities. Results from 16S rRNA analysis indicated possible functional groups of PAHs degradation include three species in *Bacillus cereus* group,*Bacillus* sp. SA Ant14,*Nocardioides sp.,*and *Ralstonia pickettii.*Principal component analysis (PCA) indicates significant positive correlations between the content of high-molecular-weight PAHs and the distribution of *Bacillus weihenstephanensis*KBAB4 and *Nocardioides sp*. The species *Bacillus cereus*03BB102, *Bacillus thuringiensis,Bacillus weihenstephanensis*KBAB4, and *Nocardioides sp.* were recognized as main PAH degraders and thus recommended to be utilized in further bioremediation applications.The vertical distribution characteristics of PAHs in soil profiles (1-12 m) and the internal relationship between the transport mechanisms of PAHs and the response of soil biological properties help further understand the microbial diversity and activity in an aged site.

MO010
BIODEGRADATION OF HYDROCARBONS BY FUNGI IN SOILS CONTAMINATED BY GASOLINE
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The infiltration of petroleum products into the soil from leaks and acidental spillages is a serious problem for the environment, since it is composed of toxic elements that have great mobility. Among them are the monoaromatic hydrocarbons that are the volatile organic compounds: Toluene, Ethylene, Benzene and Xylenes (BTEX). In view of the above, the work aimed to use two species of fungi to biodegrade the BTEX compounds in soil, analyzing their behavior, through chromatographic analysis and enzymatic activity of laccase, glycosidase and total proteins. The following species of fungi were used: *Lentinus edodes* and *Pleurotus ostreatus*. In order to verify the behavior of the fungi in soil degradation of BTEX, five contamination simulations were carried out in which there were soil contaminated, soil with a 10% nad 15% increased in *Pleurotus ostreatus,* soil with 10% and 15% increased in *Lentinus edodes*, the soil with a 15% and five simulations were carried out in soil without contamination containing the same proportion of fungi that were in soil with contamination. It was verified that the development of the fungi was quite satisfactory in the contaminated soil, developing more quickly and more intensely than in the soil without contamination. Chromatographic analysis revealed a potential mean of HC biodegradation by fungi

MO011
Evaluation of the toxic effects of sawdust used to phosphorus recovery of a eutrophic aquatic environment: a possibility to ensure global food security
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Introduction: The use of sawdust as biosorbent to phosphorous, for posterior use in agriculture, is a possible solution to ensure water and global food security through a scenario of water bodies eutrophication and phosphate rock depletion. Considering the use of phosphate enriched sawdust as an agricultural fertilizer the aim of this work was to evaluate the toxicity of sawdust after phosphorus adsorption by tests using the lettuce plant (*Lactuca sativa*) to determination the germination index and the size of the radicle and hypocotyl. To evaluate the potential risk of human infection and animals by pathogens, the determination of helminth eggs was also performed in sawdust. **Methods:** The Petri dishes containing twenty lettuce seeds at the control soils (tropical artificial soil) and at amended soils (tropical artificial soil with an addition of sawdust up to 1% (m/m)) were kept in BOD chamber at 22 °C for 120 h. The experiments were carried out in triplicate. They were watered to keep the soil at about 60% of its water-holding capacity. The germination index and the radicle and hypocotyl lengths of lettuce were determined. The determination of helminth eggs was done using the concentration method; this is a modified USEPA method using $ZnSO_4$ solution. **Results and discussion:** The results of the lettuce seed germination bioassays showed that have not impact of sawdust on germination; the germination index was the same for control and amended soils, 92.5%. The results showed that there was no significant difference ($p < 0.05$) in radicle and hypocotyl length of control and amended soils. The radicle and hypocotyl lengths for the control soil were 28.90 ± 1.01 and 35.98 ± 1.88 mm, respectively. For the amended soils were 27.18 ± 0.98 and 34.46 ± 1.61 mm, for the radicle and the hypocotyl lengths, respectively. The helminth eggs were not found in the sawdust. **Conclusion:** In this study phytotoxic effects on lettuce and infection risks to humans and animals of due the presence of helminth eggs were not identified. This reinforces the use potentiality of sawdust as a biosorbent aimed at phosphorus recovery aiming soil fertilization and plant nutrition. **Acknowledgments:** São Paulo Research Foundation, FAPESP (Process 2016/00490-6). **References:** Pantano G, Ferreira JS, Aquino FWB, Pereira-Filho ER, Mozeto AA, Fadini PS (2017), Environ Sci Pollut 24: 2685; Lutterbeck CA, Kern DI, Machado ÊL, Kummerer K. (2015) Chemosphere 135: 403.

MO012
Soil Amendments' Influence on Microbial Diversity, Pesticide Degradation and Leaching of Nutrients
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Soils have a key function in providing a multitude of ecosystem services, many of them linked to their microbial community. Sustainable soil management is necessary when aiming to preserve biodiversity on the one hand whilst maintaining productive farming on the other. Due to the importance of soils for food production, farmers are interested in soils being fertile, easy to manage, and that are efficient in water and nutrient cycling. However, pesticides and excess nutrients are major threats to the integrity of soils, and due to leaching and run-off of the pollutants, also for adjacent ecosystems. Organic matter is a structurally and functionally important part of the soil. With its diverse molecular structure the organic material is able to sorb nutrient surpluses, pesticides and their metabolites, as well as other pollutants like antibiotics from livestock. Furthermore, an increased organic matter content enhances microbial activity and subsequently improves nutrient cycling and the degradation of pollutants in the soil. Organic material also improves the air- and water balance in soil and can reduce erosion. Optimal soil conditions can even lead to a reduced need of pesticides and fertilizer, and thus contribute to a sustainable management and protection of surrounding environment in the long-term. Our aim is to investigate the influence of different organic soil amendments on microbial biodiversity, pesticide degradation and nutrient leaching in an agricultural soil. We will compare the impact of such amendments on the degradation of the radiolabeled pesticides metalaxyl and MCPA, which are used as a tank mix in potato cultures, and on the leaching of nutrients and pesticides. Microbial communities of bacteria and fungi will be analyzed in parallel via genome sequencing. Finally, based on these endpoints the most promising amendment will be tested under field conditions to assess the applicability of the laboratory results to agricultural management. **Acknowledgement** This research is supported by the Federal Agency for Nature Conservation (BfN, Germany).

MO013
The effect of biochar physico-chemical properties on the sorption behaviour of conazole fungicides
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Pesticides are an indispensable part of modern agricultural management, contributing to food security and safety. On the other hand, their extensive use may result in soil contamination as well as surface water contamination, both associated with negative impacts on non-target organisms. Thus, soil remediation amendments are sought-after to counteract the presence of contaminants in soil and/or to minimize their transfer into surface water. Biochar, the product of thermal decomposition of biomass under oxygen-limited conditions, has excellent sorption properties, which make it a valuable sorbent and a suitable alternative for treatment of contaminated soils. Moreover, biochars application to agricultural soils has been shown to increase soil fertility, mainly due to improved nutrient and carbon availability, protection of microorganisms and increased water holding capacity. However, despite these benefits, very little biochar is currently utilized as a soil amendment for either the control of contamination or for improving soil quality, mainly because the consequences of its use in soil are still not well understood. Therefore, the objective of this study was to characterize the effects of biochar physico-chemical properties on the sorption behaviour of conazole fungicides (i.e. epoxiconazole and tebuconazole) and to better understand the relationship between biochar production conditions and biochar origin and sorption of these model pesticides. For that purpose, sorption experiments were carried out using biochars produced from various biomass waste materials (e.g. rice husks, wood chips, cherry stones, chicken manure, etc.) with slow and fast pyrolysis (10 and 30 minutes) under a range of pyrolysis temperatures (300 – 750°C).We assume that apart from the mechanistic understanding of pesticide-biochar interactions, the study contributes to a better biochar management in agricultural with respect to biochar designing, production and dosing.

MO014

Humic acids impact on bioactivity of iron ions and nanoparticles

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At present the question still remains whether the toxicity of iron oxide nanoparticles (NPs) from the released ions or NPs itself arises. In this study, behaviors of Fe_3O_4-NPs and iron ions (Fe^{2+} and Fe^{3+}) in the presence of humic acids (HA), an natural organic polyligand that easily coordinate to iron (II, III) ions and graft to NPs surfaces, were investigated in the aspects of their toxicity to *Sinapis alba* and *Paramecium caudatum*. The Fe(II, III)-humic complexes were prepared by reaction of weighed amounts of the HA and the iron source with HA:Fe ratio of 1:0.150 (w/w). Colloidal Fe_3O_4-NPs were synthesized by a chemical precipitation method *in situ* when the magnetic particles are grown within the HA matrix that provides steric stabilization of the colloidal system. Several treatments of the test cultures were made: a) control A – tap water without Fe and HA; b) control B − tap water with Fe (II) or/and Fe(III), c) control C − tap water with HA, d) five treatments with the following substances: Fe(II, III)-HA: Fe(II)-HA, Fe(III)-HA, Fe(II, III)-HA, Fe_3O_4-NPs and Fe_3O_4-NPs-HA. Endpoints (EC50) and threshold level (EC20) for samples with acute toxicity have been studied in test systems with *S. alba* and *P. caudatum*. According to toxicity decreasing for both test cultures the studied samples can be arranged in the following order: Fe(II), Fe(II, III) ≥ Fe(III) >HA >Fe_3O_4-NPs-HA >Fe(III)-HA >Fe(II)-HA ≥ Fe(II, III)-HA >>> Fe_3O_4-NPs. Fe(II) and/or Fe(III) ions and HA clearly demonstrated increased toxicity with respect to root length of *S. alba* seedlings and mortality of *P. caudatum*. Combination of Fe (II and/or III) with HA lead to mitigation of toxicity. The Fe_3O_4-NPs in some concentrations stimulated the survival of ciliates and the growth of higher plants. We suppose that the observed low toxicity for Fe_3O_4-NPs is due to the released metal ions into the supernatant. In case of the Fe_3O_4-NPs-HA treatment, the disperse system effect as a whole is affected. Interpreting the results was performed by a two-way ANOVA with interactions using XLSTAT Software, 2014. The treatment method (single HA, single NPs or their mixture) and the treatment concentration have a significant effect on acute toxicity samples. For both Fe_3O_4-NPs and Fe_3O_4-NPs-HA the toxicity more largely depend on the treatment concentration. Acknowledgement. This research has been financed by the Russian Foundation for Basic Research (#18-33-01270/18) and Russian Sciance Foundation (#16-14-00167)

MO015

Weed based bioformulations to improve phytomediation of oil-polluted soils

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Screening, collection, identification and expression of stable and technological microbial strains living in the rhizosphere of weed − couch-grass *Elytrigia repens* L.were conducted. Rhizobacteria (RB) were isolated from soil samples in sanitary-protective zone of Variogan oil deposit (Tyumen region, Russia). A total of ca. 100 RB isolated were assessed for biocontrol activity *in vitro* against phytopathogenic fungi incl. *Fusarium culmorum*, *F. heterosporum*, *F. oxysporum*, *Drechslera teres*, *Bipolaris sorokiniana*, *Piricularia oryzae*, *Botrytis cinerea*, *Colletothrichum atramentarium*, *Cladosporium* sp. and *Stagonospora nodorum.* Biocontrol activity was performed using four methods: inoculation by radial and parallel streaks and by block and lawn. A culture collection comprising 64 potential biocontrol agents against wheat and barley root diseases has been established.RB isolatedhave been studied at both bioassay and laboratory vegetation experiments. All the experiments confirmed not only high antagonistic potential of those RB in relation to pathogenic fungi but also their plant growth-promoting activity. As a result, four bacterial strains were chosen as a core of pure cultures collection with high fungicidal and plant growth-stimulating potential. Partial determination of nucleotide sequence of 16S ribosomes of tested bacteria indicated that *Pseudomonas* and *Bacillus* species were the most dominant bacteria exhibiting biocontrol activity. Technology to establish liquid and dry microbiological formulations using beneficial RB has been developed. Potassium humate was used in combination with bacteria cells to protect thermolabile RB cells at concentration, drying and long-term storage. Humic substances have proved to be an excellent matrix for immobilization of cells due to its biocompatibility, non-toxic nature, reduced vulnerability to environment stresses which will provide prolonged action of bacterial preparation in field conditions. Biological tests on bioproduct quality towards barley seeds demonstrated an increase in the dry weight of shoots, roots, total biomass to 20, 80, and 30 % accordingly in comparison with the control (no inoculate). Obtained results demonstrated that approach applied was promising in terms of producing weed-isolated RB enriched with humics. Further study aiming to estimate its oil-degrading properties and evaluation of its production at industrial scale are needed.

MO016

Sorption of ciprofloxacin using humics-coated Fe3O4 nanoparticles

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In recent decades, water quality became a crucial issue also in developed countries due to identification in surface and drinking waters of new classes of pollutants, which were previously ignored. Among them antibiotics are of great concern due to their increased consumption, long-term stability in water and soil, and poor efficiency of recovery at conventional water treatment facilities. Ciprofloxacin (CIP) is a fluoroquinolone antibiotic with a broad spectrum of action, which is more often found in surface and wastewaters as compared to other antibiotics. One of the most commonly used method for the removal of CIP from wastewater is adsorption. This study is aimed to estimate surface-dependent effect of humics-conjugated magnetite (Fe_3O_4-HA) on sorption of ciprofloxacin. The nanoparticles of magnetite were synthesized *in situ* by oxidative alkaline hydrolysis of the iron (II, III) powder precursors into humics medium. The synthesized particles prepared at different humics concentration (10, 20, 40 wt%) were characterized using X-ray powder diffraction (XRD), scanning electron microscopy (SEM), Mossbauer and FTIR spectroscopy, DLS and BET analysis. The CIP recovery was studied from 0.1 mM solution at solid:liquid ratio 1:1000, pH 7.5, and the contact time 24 h. The equilibrium concentration was determined spectrophotometrically. The results showed that the size and specific surface of nanoparticles depended upon the humics concentration. XRD results indicate that the addition of HS into Fe_3O_4 nanoparticles media have not changed the crystal structure of nanoparticles, but the intensity of the peaks is reduced with using of HA. The average crystal size of the nanoparticles calculated from the diffraction peak half-widths according to Scherrer's equation for the Fe_3O_4-HA in concentrations of 10-40 wt.% decreased from ~17 nm to ~10 nm, respectively. Moreover, Fe_3O_4 conjugation with humics lead to increase specific surface from 117 to 142 m^2/g for the Fe_3O_4 and the Fe_3O_4-HA20 accordingly. The sorbents under study are characterized by a high sorption capacity regarding to ciprofloxacin. The results obtained indicate that the adsorption of CIP by the Fe_3O_4-HA20 is largely enhanced in comparison with the bare Fe_3O_4. Acknowledgement. This research has been financed by the Russian Foundation for Basic Research (#18-44-920007 – synthesis and characterization of composites, and #18-33-00335 mol_? – investigations of CIP sorption).

MO017

Effect of sodium hexa-metaphosphate on soil hydraulic properties

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In the field of soil remediation, removal of the contaminants largely depends on the soil permeability. Number of research have been conducted on infiltration and assisted remediation. Aggregation of the soil particle in the soil due to ionic attraction makes the soil less permeable for infiltration. However, very little action has been employed for the dispersion of the clay particle and assisted infiltration in the soil. This study would like to investigate different soil- hydraulic properties and associated infiltration of water in the partially saturated porous media with sodium hexa-metaphosphate (Na-HMP). Three solutions (water, 0.1% Na-HMP and 8.0% Na-HMP) were used for the premixing process. Soil was mixed with solutions and left overnight. Cumulative infiltration was recorded from a soil column equipped with the Marriott reservoir and mini disk infiltrometer. Significant difference was found for the test of infiltration and unsaturated hydraulic conductivity at a significance level of $\alpha = 0.05$. From the mean value comparison, differences was investigated for the soil treated with the water and Na-HMP. Highest difference was observed between 8% Na-HMP and water. With the addition of small concentration of Na- HMP, significant improvement in the soil-hydraulic properties was obtained. Improved soil-water property equipped with the infiltration using Na-HMP was due to the dispersion of the clay particle associated with high zeta potential and apparent reduction in the viscosity of the solution. (This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government (MSIT) (No. 2018R1A2B6006139))

Biogeochemistry and Fate of Organic Pollutants in Aquatic Systems (P)

MO018

Trends in mercury pollution levels of aquatic ecosystem components in the Republic of Uzbekistan

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Determination of total mercury in filtered water samples was conducted by cold-vapor atomic-absorption method. Mercury concentrations in samples of aquatic bottom sediments and fish muscle from Uzbekistan were determined in CNR-IRSA, Italy using atomic-absorption spectrometry (AMA 254). The analyses of mercury concentrations in river waters during 2004-2014 have revealed that, in most rivers, the concentration of mercury ranged between below detection limit (0.02 μg/L) and 0.35 μg/L which does not exceed all existing MPC for drinking water. However, these values exceeding MPC for natural waters established by EU directive 2013/39 (0.01 μg/L). Mercury concentrations found in some rivers such as Chirchiq, Akhangaran, Zarafshan, Syrdarya and Amankutansay, as well as canals (i.e. Salar, Bozsu and Siab), have reached 0.1 μg/L and even higher levels in some years. Concentrations higher than 0.1 μg/L can be considered as ecologically dangerous also due to possible analytical errors and increasing emissions. Concerning pollution sources as a rule, these rivers are situated in, or running through, industrialized and urbanized areas. Only two rivers may be considered as effected by transboundary pollution from neighbouring Kyrgyzstan and Tajikistan. Mercury concentrations detected in bottom sediments (maximum 26±2.8 μg/kg) and fish muscles (range 28-138 μg/kg) were much lower than MPL for soils – 2100 µg/kg and fish for human consumption (300 µg/kg). Levels in fish were however higher than the environmental quality standard of 20 μg/kg established by the European Union for the protection of aquatic wildlife against secondary poisoning of top predators such as aquatic birds and mammals. Based on conducted investigations it may be concluded that past and present environmental mercury pollution rates in Uzbekistan were within the permissible levels for human health (drinking water). However existence of possible dangerous impact of industrial and urban contamination on natural aquatic ecosystems reflected by elevated concentrations (higher than 0.1 μg/L Hg) indicates an urgent need to start future investigations in industrialised regions of the country and neighbouring Republics where large sources of mercury pollution exists.More extensive studies on Hg levels in fish and other organisms may be needed to understand if current Hg levels can pose a risk to top predators.

MO019

Water and sediment chemistry influences on mercury bioaccumulation in freshwater invertebrates from two lakes in Kejimkujik National Park, Nova Scotia

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Mercury is a trace metal and a toxic environmental pollutant that can be deposited in remote ecosystems and result in adverse effects on the heath of organisms. Elevated mercury levels at the bottom of the food web have implications for significant mercury (in the form of methylmercury (MeHg)) transfer to higher trophic organisms. Kejimkujik National Park (KNP), Nova Scotia is an area with a high landscape sensitivity to interactions with mercury. MeHg bioaccumulation in KNP lakes is of high concern in the area because elevated MeHg near the bottom of the food chain, such as near-sediment invertebrates, has implications for significant mercury transfer to higher trophic levels. Therefore, analysis of MeHg concentration in aquatic invertebrates and the relationship with water and sediment characteristics is required to understand food web bioaccumulation and relative bioaccumulation between invertebrates. Mayfly naiads, caddisfly larvae, lake water, and benthic sediments were collected from two lakes and one wetland catchment in KNP. Methylmercury (MeHg) was analyzed in all samples using gas chromatography-atomic fluorescence spectroscopy (AFS). MeHg concentrations in mayflies and caddisflies ranged from 14.28–427.78 ng/g (N=58), 0.05–0.46 ng/L (N=45) in water, and 0.02–28.94 ng/g (N=22) in sediment. It was found that mayfly MeHg concentration was positively correlated with water MeHg concentration (Spearman Rank correlation coefficient (r_s)=0.43; P-value=0.02). Caddisflies may be more associated with sediment MeHg concentration than water MeHg (r_s=0.45;0.07, respectively), although correlations were not significant. Most sediment and invertebrate samples and all water samples from the wetland catchment were significantly higher in MeHg concentration than the lake locations because wetlands are areas of efficient MeHg production. In conclusion, no significant relationships were observed between MeHg concentration in caddisfly and mayfly and sediment MeHg. However, our data do suggest that mayfly MeHg concentration is influenced by water characteristics (MeHg, pH). Analysis of MeHg bioaccumulation in aquatic invertebrates and the relationships with water and sediment characteristics are key. More research into MeHg bioaccumulation is necessary to better understand food web transfer.

MO020

Using laboratory incubations to predict the fate of pharmaceuticals in aquatic ecosystems

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Environmental persistence is a key property when evaluating risks with excreted pharmaceuticals in aquatic ecosystems. Such persistence is typically predicted using small-scale laboratory incubations, but variation in aquatic environments and scarcity of field studies verifying laboratory-based persistence estimates create uncertainties around the predictive power of these incubations. In this study we: i) assess persistence of five pharmaceuticals (diclofenac, diphenhydramine, hydroxyzine, trimethoprim and oxazepam) in laboratory experiments under different environmental conditions; and ii) use a three-month long field study in an aquatic ecosystem to verify the laboratory-based persistence estimates. In our laboratory assays, we found that water temperature (TEMP), concentrations of organic solutes (TOC), sediment presence (SED), and solar radiation (SOL) individually affected dissipation rates. Moreover, we identified rarely studied interaction effects between the treatments (i.e. SOL x SED and TEMP x SOL), which affected persistence of the studied drugs. Half-lives obtained from the laboratory assays largely explained dissipation rates during the first week of the field study. Yet, none of the applied models did accurately predict long-term dissipation rates (monthly time-scale) from the water column. For example, the studied antibioticum (trimethoprim) and the anti-anxiety drug (oxazepam) remained at detectable levels in the aquatic environment long after (~150 days) our laboratory based models predicted complete dissipation. We conclude that small-scale laboratory incubations seem sufficient to approximate short-term (i.e. within a week) dissipation rate of drugs in aquatic ecosystems. Yet, this simplistic approach does not capture interacting environmental processes that preserve a fraction of dissolved pharmaceuticals for months in natural water bodies.

MO021

Occurrence of Pharmaceutical and Personal Care Products in Wild Fish Plasma in Taihu Lake and Application of the Fish Plasma Model

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Pharmaceuticals and personal care products (PPCPs) are a class of emerging contaminants and occur widely in aquatic environment at low concentrations ranging from ng/L~µg/L. Long-term exposure to low concentration of PPCPs may pose potential toxicity to fish as well as other aquatic organism, thus harm to aquatic ecosystem. Analysis of plasma concentration of PPCPs in wild fish which is the typical spinal aquatic organism is of great significance. The Fish Plasma Model use the drug concentration in fish plasma to estimate the ecological risk of the pharmaceuticals to aquatic organisms. However, the existing fish Plasma model(log $P_{\text{Blood: Water}}$=0.73×Log K_{OW}-0.88) is not yet perfect, which is mainly comfit for hydrophobic substances. Taihu basin is located in the Yangtze River Delta economic zone in China. The industrial production and population density in Taihu basin is so high that the production and consumption of PPCPs in this area account for the high proportion of the whole country. Typical wild fish include crucian carp, bighead carp, silver carp, common carp, surf fish, carassius auratus and hemiculter clupeoides in Taihu Lake were study from 2015 to 2017, and their plasma samples were analyzed for 29 PPCPs. In general, the detection concentration of PPCPs in the plasma of wild fish was higher than that in the environment, ranging from 0.13 to 2824.21 ug/L. In three years, 25 kinds of PPCPs were detected in all fish plasma except ENR, CTC, SMZ and GFZ. From the distribution of fish species, crucian carp had the highest plasma concentration, followed by bighead carp, silver carp and common carp. The concentration of PAR in plasma was the highest, ranging from 2.17 to 717.03 ug/L. Because of the large difference between K_{ow} and D_{ow} of most substances, the original fish plasma model can not be directly applied to all drugs. For improvement, we replace K_{OW} with D_{OW}, introduce the half-life of the drug, classify the drugs according to different drugs or different properties to obtained a better fitting formula (log $P_{\text{Blood: Water}}$ = a + b × log D_{OW} + c×exp(d×t1/2)). Based on the improved fish plamsa model, the trend of BAF in measured fish plasma is the same as that in predicted $P_{\text{Blood: Water}}$. The predicted steady plasma concentration in fish is generally lower than the measured value, which indicates that the ecological risk calculated by the formula is seriously underestimated.

MO022

Impacts of Bacterial Diversity and Hyporheic Exchange Flows on the Fate of Wastewater-derived Polar Organic Micropollutants - A Central Composite Face Designed Flume Study

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The combined effects of bacterial diversity and hyporheic exchange flows on the fate of 30 polar organic micropollutants were investigated in 24 recirculating flumes using a fractional factorial design. The 2 meter long sediment and water filled outdoor flumes were sheltered from precipitation and direct radiative forcing to control water and energy in- and outputs. Polar organic micropollutants were fortified at 10 μg/L each, and surface water and pore water concentrations were tracked over 78 days. Bacterial diversity (low, medium, or high) was adjusted by diluting sediments from a wastewater-impacted stream, while different intensities of hyporheic exchange flows (low, medium, or high) were obtained by adjusting the number of bedforms of the flume sediments. Nutrient concentrations, pH, dissolved oxygen profiles, solar radiation, water level, bacterial diversity, and temperature were monitored throughout the course of the experiment. Each combination of variables (i.e. bacterial diversity and hyporheic exchange flow) was replicated once for model development, after which validation of the model was carried out using n=4 mid-point experiments. Two non-inoculated and two non-spiked controls were included to quantify abiotic losses and background contamination, respectively. Dissipation half-lives (DT50s) were fitted to a response surface model of the parameters bacterial diversity and hyporheic exchange flow. Our initial hypothesis was that increased bacterial diversity and hyporheic exchange flow would result in a decrease in DT50s. However, while DT50s of almost all compounds decreased with increasing bacterial diversity, the effect of bedform-induced hyporheic exchange was less marked. Pharmaceuticals such as valsartan, propranolol or sotalol seemed more stable in flumes with a high number of bedforms. The fate of the anti-diabetic drug metformin was unaffected by altered hyporheic flow but was almost fully transformed to guanylurea and partially further degraded within 28-42 days in flumes with high bacterial diversity. A number of transformation products such as metoprolol acid, carbamazepine-10,11-epoxide and valsartan acid originating from other parent compounds were also detected. This study provides evidence that hyporheic exchange flow and especially bacterial diversity are important factors of polar organic micropollutant degradation in stream systems.

MO023

Metabolites & transformation products of medicinal products - are they of environmental relevance?

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Pharmaceuticals administered to the patient, be it human or animal, are metabolised in the body and are excreted as parent compound and/or metabolites. Hence, in contrast to other substance classes, pharmaceuticals enter the environment almost entirely as a mixture of parent and metabolites and may be further transformed during activated sludge treatment in sewage treatment plants and/or in the receiving environmental compartments. However, in existing risk assessment concepts, the estimation of the predicted environmental concentration (PEC) is primarily based on the so-called total residue approach, i.e., metabolism in patients is not taken into account. The basic idea of this concept is that it is assumed that neither metabolites nor transformation products have a greater impact on the environment than the parent compound. However, it remains debatable whether this approach is valid. For instance, the fact that individual excreted metabolites or resulting transformation products may be more hydrophilic than the parent compound and hence may be more mobile and/or more persistent in the environment needs to be considered e.g. with respect to groundwater protection. In such cases, metabolites and/or transformation products might be even more relevant than the parent compounds. Based on long-term experiences in environmental risk assessment within the authorisation procedure of human medicinal products the poster will present insights on fate and potential environmental impact of metabolites and transformation products of active pharmaceutical substances. According to the ‘Guideline on the environmental risk assessment of medicinal products for human use’ (EMEA/CHMP/SWP/4447/00, corr. 2) the fate of an active substance in the environment is described with a study on adsorption /desorption (OECD 106, Koc), a study on ready biodegradability (OECD 301) and a water-sediment simulation test (OECD 308). Furthermore, for some substances with a Koc-value > 10.000 also a study on aerobic transformation in soil (OECD 307) should be available. Based on these scientific findings the poster provides a critical reflection on the relevance of metabolites and transformation products of medicinal products in the light of current risk assessment strategies.

MO024

Effects of antibiotic residues on the growth and photosystem II efficiency in baltic cyanobacteria

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The aim of the study was to measure the influence of selected antibiotics, most often detected in the Baltic Sea, on the growth and photosystem II of non-target organisms - cyanobacteria *Microcystis aeruginosa* and *Nodularia spumigena* and the model organism *Chlorella vulgaris* by using longer lasting tests (11 days). Standard measurements (cell number, optical density, chlorophyll *a* concentration) were combined with OJIP fluorescence transitions measurements. The obtained results showed that low, theoretically safe levels of antibiotics residues can have negative impact on the tested microorganisms. Oxytetracycline caused decrease of photosystem II efficiency and showed the potential to disrupt photosynthesis process

MO025

MICROBIAL RESPONSES TO ANTHROPOGENIC DISSOLVED ORGANIC CARBON IN ARCTIC AND ANTARCTIC COASTAL SEAWATERS

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Thousands of semi-volatile hydrophobic organic pollutants (OPs) reach open oceans through atmospheric deposition causing a chronic and ubiquitous pollution by anthropogenic dissolved organic carbon (ADOC). Besides be prone to biodegradation, these compounds accumulate in cellular lipids, inducing harmful effects on marine biota. Unfortunately, their possible effects on microorganisms, key drivers of global biogeochemical cycles, remain unknown. We challenged coastal microbial communities from Ny-Ålesund (Svalbard, Arctic) and Livingston Island (South Shetlands, Antarctica) with ADOC amounts within the range of oceanic concentrations in 24 hours incubations to investigate this largely uncharacterized vector of global change. ADOC addition elicited clear transcriptional responses in multiple microbial heterotrophic activities in ubiquitous groups such as Flavobacteriia, Gammaproteobacteria and SAR11. Importantly, a suite of cellular adaptations and detoxifying mechanisms, including remodeling of membrane lipids and transporters, was detected. ADOC exposure also changed the composition of microbial communities, in particular by the increased number of 58 taxa in Arctic and 34 taxa in Antarctica from the rare biosphere. Many of these taxa belong to described OP-degrading bacteria and showed the most different gene expression profiles between ADOC amendments and controls among all groups. This work shows that ADOC at environmentally relevant concentrations substantially influence the marine microbial communities.

MO026

Measuring aerobic biodegradation kinetics of organic micropollutants in-situ

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Accurate assessment of the biodegradation kinetics of organic micropollutants is essential to characterize their behavior and persistence in the environment. The OECD 309 test for the assessment of aerobic mineralization of organic micropollutants such as pharmaceuticals in surface waters has shortfalls that reduce its environmental relevance. Studies have shown that the concentration of spiked analytes, often well above environmental concentrations in tests, significantly affects the observed biodegradation rates. Likewise, spiked samples typically exhibit a lag phase, during which the microbial population undergoes adaptation, departing from steady-state natural conditions. Targeted analysis of spiked samples limits the range of chemicals tested and the requirement of a laboratory environment limits the range of water sample locations, and hence the range of microbial populations. A prior study has demonstrated that biodegradation can be measured in unspiked natural water samples. To further increase the environmental relevance of these tests, we again use unspiked natural water but instead perform the kinetics study in-situ. By suspending amber glass bottles as our test chambers in a river downstream of a wastewater treatment plant, we parallel the natural system more closely than a lab test and simultaneously reduce the resources necessary, enabling these tests to be carried out in more remote areas. We compare our results to those of an unspiked laboratory biodegradation test, using non-target high resolution mass spectrometry to quantify biodegradation kinetics for a broader range of micropollutants.

MO027

Influence of sediment bacterial community composition on biotransformation of organic micropollutants

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Biodegradation is an important removal mechanism of micropollutants in aquatic ecosystems and comprises multiple processes which are performed primarily by bacteria. To assess biodegradation in water-sediment systems, the OECD308 standard test is often used. For this study we hypothesize that different sediment substrates may contain different bacteria communities and its composition can influence the degradation of micropollutants. We performed bottle incubations of ten micropollutants: acetaminophen (ACT), acesulfame K (ACK), caffeine (CAF), carbamazepine (CBZ), diclofenac (DIC), furosemide (FUR), metformin (MET), oxazepam (OXZ), tramadol (TRM) and venlafaxine (VFX) according to the OECD308 standard using sediment from rivers Fyris (F), Sweden and Gründlach (G), Germany, before (1) and after (2) the discharge of a local waste water treatment plant (WWTP). The dissipation half-lives (DT50s) were calculated assuming first order kinetics. Illumina sequences of bacterial 16S rRNA were used to analyse the bacterial community composition in the sediment at the end of the incubation. The DT50s obtained for five micropollutants (CBZ, OXZ, TRM, VFX and ACK) have significant differences (p-values ≤ 0.05) between the two rivers and locations to the WWTP, DIC presented differences between rivers and FUR between locations. Except for ACK, longer DT50s were observed at location G2 than in any other treatment, and shorter in G1 and F1. The analysis of sequences indicate that there are no significant differences in the structure of the bacterial communities in sediment from F, but that the two locations in river G are significantly different from F (p-value ≤ 0.001) and each other (p-value ≤ 0.001). Differential abundance analysis for DT50s of CBZ, VFX and TRM, show that 58 bacteria genera are significantly more abundant in the samples where fast degradation was observed and could be associated with this response. Slow degradation was associated with 32 bacteria genera for the same four compounds. Different genera were significant for the differences in fast or slow degradation of OXZ, DIC and ACK. Random forest discriminant analysis as well as partial least square discriminant analysis were also performed for comparison. This study shows that bacteria community composition can influence the observed degradation of micropollutants in OECD308 test and that specific bacteria consortia might be associated with fast or slow degradation.

MO028

The Biodegradability of Acetaminophen using Multiple OECD 301 Guidelines and the OECD 307 Guideline

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The ready biodegradability of Acetaminophen is assessed in this study based on several OECD 301 Guidelines and its biodegradability in the natural environment is determined using a simulation test based on the OECD 307 soil metabolism guideline. Several studies are publicly available that show Acetaminophen being biodegradable in activated sludge. However, most of these studies do not follow the strict requirements of the OECD 301 Ready Biodegradability Guidelines. This study aims to determine if Acetaminophen can pass a Ready Biodegradability Test and if the results are repeatable using several different test designs permitted by the OECD 301 Guidelines. The exact OECD guidelines followed were the OECD 301B, OECD 301F and OECD 310 Guidelines. The ready biodegradability results for Acetaminophen, based on CO_2 evolution, obtained in the OECD 301B portion of the study and average CO_2 evolution results obtained in the soil metabolism study using two soils are presented below: **Ready Biodegradation, %** Days 1 2 5 9 16 28 Acetaminophen 0.4 -1.0 45.7 61.9 70.5 67.5 **CO_2 Evolution from Soil, %AR** Days 4 15 33 64 123 Acetaminophen 13.0 16.3 19.8 23.5 26.5 Results from additional tests, using the other 301 guidelines, will be presented and compared to the OECD 301B results. Reasons for the differences of the CO_2 evolution results between the 301 studies and the soil simulation test, will be discussed in relationship to non-extractable and bound residues. The use of Acetaminophen as a potential reference compound will also be evaluated.

MO029

Transformation of organic chemicals in aquatic sediment systems (OECD 308) under simulated natural sunlight

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Chemicals that are directly applied to surface water or that are likely to reach the aqueous environment by routes such as run-off, drift and agricultural effluents, are required to pass environmental risk assessments. As an essential part of tiered testing strategies, the OECD 308 Guideline defines laboratory testing to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems in the dark. In order to further simulate environmental conditions, and ascertain half-lives or disappearance times (DT50, DT75, DT90) for test compounds while being exposed to irradiation, requests for including natural sunlight and/or Algae in OECD 308 study designs have been emerging. Innovative Environmental Services (IES) Ltd, Switzerland, as an independent GLP-certified contract research organization, brings ten years of experience with aerobic aquatic sediment studies under simulated natural sunlight conditions, performed for a variety of recurring clients. The laboratory set-up used by IES Ltd for irradiated OECD 308 studies, with and without Algae, will be elaborated upon in detail, and potential advantages and disadvantages of including simulated natural sunlight in the test design will be explained. In addition, the need for a new or adapted test guideline will be discussed.

MO030

Natural Bromoanisoles in Northern Baltic Estuaries

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Bromoanisoles (BAs) are O-methylation products of bromophenols (BPs), which are produced by many species of marine macroalgae [1] and cyanobacteria [2]. BAs evaporate from the sea [3] and are transported long distances through the atmosphere [4]. BAs and BPs bioaccumulate, from macroalgae to grazing amphipods to fish [5]. In our investigations of macroalgae as a source of BAs to Baltic ecosystems [1], we measured BAs in seawater of four estuaries and at two offshore stations in the northern Bothnian Sea (northern Baltic) at monthly intervals during spring-summer, 2018: late May-early June, mid-July and mid-August. Only 2,4-DiBA and 2,4,6-TriBA were found; those with meta-Br substitutions were absent. Over all months, the concentrations of 2,4-DiBA (63-457 pg L^{-1}) and 2,4,6-TriBA (157-768 pg L^{-1}) were higher in estuaries than offshore (43-78 and 86-143 pg L^{-1}, respectively) Monthly variations in the estuaries were strikingly different for the two compounds: 2,4-DiBA peaked in mid-July, with lower concentrations in May-June and mid-August, while 2,4,6-TriBA was highest in May-June and declined over the summer. Offshore trends mirrored those in estuaries, but with lower amplitude. Similar concentrations were observed in August at the island Holmön, where 2,4-DiBA and 2,4,6-TriBA were 340 and 400 pg L^{-1} in a shallow strait with visible macrophytes versus 53 and 102 pg L^{-1} at an offshore station. Concentrations and monthly variations of the two BAs are likely due to differences in macrophyte and/or cyanobacteria populations, rates of O-methylation which converts BPs to BAs, and to estuarine-offshore water mixing. [1].Bidleman, T.F. et al., 2019. Natural brominated compounds in Nordic macroalgae. SETAC-2019, Helsinki [2] Löfstrand, K. et al., 2010. Brominated phenols, anisoles, and dioxins present in blue mussels from the Swedish coastline *Environ. Sci. Pollut. Res. 17*, 1460-1468 [3] Bidleman, T.F. et al., 2016. Sea-air exchange of bromoanisoles and methoxylated bromodiphenyl ethers in the northern Baltic. *Mar. Pollut. Bull. 112*, 58-64. [4] Bidleman, T.F. et al., 2017. Atmospheric transport and deposition of bromoanisoles along a temperate to arctic gradient. *Environ. Sci Technol. 51*, 10974-10982. [5] Dahlgren, E. et al. 2016. Trophic transfer of naturally produced brominated aromatic compounds in a Baltic Sea food chain. *Chemosphere 144*, 1597-1604.

MO031

Compounds of emerging concern in a changing climate: the case of 1,4-Dioxane

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Compounds of emerging concern are entering the aquatic system via various routes and are being discharged by streams and rivers eventually ending up in the ocean. At the same time we are facing the effects of climate change. Due to climate change long periods of drought can occur. This affects the water discharge and as a result also the water quality of rivers. The Netherlands, as the rest of Western Europe, have faced extreme drought in 2018. The water discharge of our rivers was historically low. In September 2018 upstream in the river Rhine 1,4-Dioxane was discharged in amongst others a small river entering the river Rhine. This had led to an exceedance of the alarm values for this compound at the monitoring stations. When a compound is found at a level above the alarm value, in this case 3 μg/l, a model is run to predict when the compound might reach the drinking water intake point. Usually this is within a couple of days. However in this case the discharge of the river was historically low whereby the model was unable to correctly predict the time at which the water intake points would be reached. Calculations had to be made by hand. Because of the low discharge the compound remained in the system much longer than during average discharge which might cause negative effects to the aquatic ecosystem. Drinking water companies producing drinking water from river water were alarmed and had to request for a temporary dispensation for water intake for a long period of time. That means that the can continue to produce drinking water even though the concentration of a compound, in this case 1,4-Dioxane, is slightly above their guideline value (1 μg/l for this compound). Continuous updates of the concentrations of 1,4-Dioxane were provided to drinking water companies. The monitoring program for 1,4-Dioxane was extended further downstream in the river and the Amsterdam-Rhine channel where concentrations were measured three times per week during a period of at least six weeks. The poster will highlight the concentrations of 1,4-Dioxane in time and space. Furthermore the concentrations will be related to river discharge. It is expected that in our changing climate we will be facing low river discharge more often. This means that in the future we will have to adjust the models to be able to adequately predict when compounds that exceed alarm values will reach the drinking water intake points.

MO032

Brominated flame retardants at the snow, seawater and plankton collected at the Antarctic Peninsula

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Concurrent sampling of seawater, snow and plankton for a range of recently regulated brominated flame retardants (BFRs), such as PBDEs, non-BDE flame retardants such as dechloranes (plus and anti isomers) and hexabromocyclododecane (α-HBCDD) were undertaken at coastal areas of Livingston Island (Antarctica). Sampling were performed during three months, covering late spring (December 2014) to late summer (February 2015) in order to assess for the first time the presence of these chemicals in the area. For seawater and plankton sampling, two locations were selected, one close to a glacier, which gives potential inputs of snow/ice to the ocean and therefore of pollutants and a second site located away from the glacier. Overall, all BFRs were detected in all the selected matrices. ΣPBDE concentrations (sum of 46 congeners, excluding BDE209) in plankton were in the range of 23-215 ng/gdw in samples collected away from the glacier. Slightly higher ΣPBDE were found in those samples collected at the glacier, with Σ_{46}PBDE concentration in the range of 32-314 ng/gdw. BDE-47 and -99 were the predominant congeners quantified in plankton samples. Overall, slightly higher concentrations of individual PBDEs (e.g 47, 99, 100, 153) were found in plankton collected close to the glacier in comparison to those samples collected far away from it, which may suggest that the melting of the glacier may be impacting the lower levels of the Antarctic food web. On the other hand, the most frequently detected non-PBDE flame retardants were dechloranes (plus and anti isomers).Dechlorane plus and anti were found in 100% of the samples analyzed for this chemical), while α-HBCDD were found in the 67% of the samples analysed. However, no differences were found in plankton collected at both selected locations.

MO033

Analysis method development of organophosphate using liquid chromatography-tandem mass spectrometry in Great Lake fish

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Flame retardants are chemicals that inhibit combustion and are added to products such as plastics, textiles and electronics. Previously used brominated flame retardants were phased out because of adverse effects in environment and health impacts on human, and replaced to alternative chemicals, such as organophosphate flame retardants (OPFRs). They are widely used for textile manufacture as easy to handle and are cost effective to recycling the products. In spite of large chemical usage, analysis information for organophosphate is insufficient. Effective analysis for OPFRs were developed to apply for Great Lake fish using liquid chromatography-tandem mass spectrometry (LC-MSMS). Twenty-two OPFRs were exposed in fish samples, *Oncorhynchus mykiss* and *Menticirrhus saxatilis*. Prepare homogenized fish samples, solvent mixture (methanol, water and formic acid) shake with fish samples. In this study, extraction steps were simplified in order to minimize chemical loss. LC-MSMS analysis method not only increased intensity of individual organophosphate chemicals, but also separated isomers well. The average recoveries were 69-140% in *Oncorhynchus mykiss* and 71-132% in *Menticirrhus saxatilis*, and limits of quantitation (LOQs) were 0.03-0.44ug/L and 0.01-0.76ug/L, respectively. Developed method were applied for fish samples in Lake Ontario, Canada and EHDP, TBEP, TCPP, TCEP and TOTP were mainly detected.

MO034

Can forest fires be a diffuse source of contaminants (PAHs) to the aquatic system?

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Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants, which have been raising important health and environmental concerns due to their toxic, carcinogenic, mutagenic and teratogenic properties. Additionally, they show a high environmental persistence and bioaccumulation potential, being some of them listed as priority pollutants by the United States Environmental Protection Agency (USEPA) and the European Commission (EC). Forest fires have been identified as an important source of PAHs in the environment. For instance, these contaminants could be mobilise during a wildfire from ashes and sediments loads and may eventually reach the nearby aquatic systems by surface runoff. These detrimental off-site effects are particularly notorious during the initial post-fire period, although not necessary limited to it. Hence, the present study aimed to assess the effects of wildfires on PAHs contamination of water resources. To this end, five campaigns, following a wildfire in north-central Portugal and during eighteen months, were performed to assess the temporal trends and profiles of the fifteen priority PAHs (by USEPA) in samples of surface runoff collected in an unburnt and burnt eucalypt plantations, as well as a nearby downstream. The principal results obtained in this study were the following: (1) immediately after the fire, the total sum of the PAHs was 4-fold higher in the burnt surface runoff than in the unburnt runoff, while the stream presented higher levels than the unburnt runoff; (2) The concentrations of the PAHs decreased gradually and approach the background levels eighteen after the fire; (3) The levels of light PAHs with three to four rings were at higher levels than heavy PAHs with five to six rings, thus showing a similar profile between the water samples; (4) in terms of individual PAHs, the unburnt runoff was dominated by the anthracene while the burnt runoff and stream water were dominated by the phenanthrene. The present study clearly demonstrated the effects of wildfires on PAHs mobilisation by overland flow and thus pointed towards recently burnt forest areas as a potential source of diffuse environmental contamination of aquatic ecosystems. This information is of crucial importance to predict the risks and effects of the surface water contamination by recently burnt areas and increasing knowledge on water quality alterations due to forest fires.

MO035

Polycyclic aromatic hydrocarbons (PAHs) in cores from the depth part of the Gulf of Mexico: an interpretation

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The Gulf of Mexico is the largest marginal se along the atlantic in the american continent. It is very important sea because of its large biodiversity and also because of its large economic contribution to both US and Mexico due to its oil deposits. It has become one of the most studied semienclosed seas after two of the largest oil spills occurred in this Gulf, the second largest the Ixtoc spill that occured in 1979 and the largest one, more recent the Macondo well which occurred in 2010 from the Deepwater Horizon (DWH) platform. It has been also registered that there are many natural filtration of oil in this part of the world oceans. There is renewed interest for understanding the functioning of the Gulf, the circulation, largely dominated by the Loop Current due to the latest spill, in particular to determine if a portion of the spilled oil reached mexican waters and sediments. Because of importance of polycyclic aromatic hydrocarbons as a relatively large component of crude oil and specially due to its larger resistance to degradation, we decided to investigate the characteristics of these compounds in several deep cores strategically located in the area of the Gulf. The importance of these chemicals is; in addition to its toxicity, their potential to help in explaining their origin using some ratios and mixed plots. This region receives a large amount of hydrocarbons, including potentially toxic polycyclic aromatic hydrocarbons (PAHs) from a several sources; river discharges, coastal erosion, atmospheric deposition, natural oil seeps, and accidental oil leaks related to petroleum exploration/transportation. The PAHs have relatively low aqueous solubility get readily sorbed onto soil and sediments, and eventually deposited to the seafloor. The seafloor sediments are considered to be the long term repository of PAHs and the deep-sea sediments are therefore the major global sink for PAHs entering into ocean waters. Two cores located in opposite zone were analyzed, one is located at the center of the Gulf of Mexico (core A5) with depth of 3,515m and a second one is located south of the Gulf of Mexico (core G44) in front of the mexican states of Tabasco and Campeche located near the oil maritime platform (Campeche Shelf) at a depth of 2,354m. Result indicate that the concentrations of ∑PAHs vary between 95 and 155. ng/g and 73.78 and 537.23 ng g-1, respectively.

MO036

Influence of stereochemistry on the partitioning of selected ortho and non-ortho polychlorinated biphenyls between aqueous solution and soil system.

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The influence of sorbate characteristics such as stereochemistry on the partitioning of selected non-ortho and ortho-substituted polychlorinated biphenyls (PCBs) between aqueous solution and soil collected along the river course of uMngeni River of KwaZulu-Natal, South Africa located in sub-Sahara Africa was investigated in this study. Specifically, PCB 77 and PCB 52 were selected in this study to represent the non-ortho and ortho-substituted system. These congeners were chosen due to their historical environmental problems and varying degree of hydrophobicity characters despite their equal numbers of chlorine atoms. Soil physicochemical characteristics were quantified such as soil organic matter, cation exchange capacity, surface area, pore volume distribution, and pore diameter as well as various functional groups present in the soil humic materials using Walkley Black, BET adsorption-desorption, and FTIR methods respectively. Batch adsorption experiments were used for the sorption studies. The results of the investigation revealed that non-ortho substituted congener was found to show more preference for soil compared to its di-ortho substituted counterpart congener of the same homolog. The effect of initial concentrations on both revealed that low initial PCB concentrations were mostly favoured for the sorption while sorption was generally low for ortho-substituted PCBs at high concentration. This could be due to the soil interlayer pore blockage which may be influenced by the position of the chlorine. Also, non-ortho PCB 77 was said to be more hydrophobic due to the orientation of chlorine atoms which allows for the free rotation of chlorine atoms between the phenyl rings and practically impossible in PCB 52 thereby making it more susceptible to aqueous solubility. Due to the susceptibility of ortho-substituted congeners to aqueous solubility, it is, therefore, more likely for this pollutant to re-suspended back to surface water or leach to the groundwater more easily when sorbed by soil and may possibly pose more risk to the aquatic ecosystem relative to its non-ortho substituted congener of the same homolog. However, PCB 77 are more likely to result in steady sorption by soil and become less available to the aquatic life which may then become more available to filter feeder organisms who source their food from surface sediment.

MO037

Diffusive air-water exchanges of Polycyclic Aromatic Hydrocarbons in an

oligotrophic North Patagonian lake from Chile

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Polycyclic Aromatic Hydrocarbons (PAHs) are semivolatile organic pollutants of concern because of their ubiquitous occurrence, bioaccumulation potential and toxic effects on ecosystems and human health. This study aim is to know the influence of local sources of anthropogenic PAHs (i.e., agricultural waste burning, indoor firewood burn and vehicular emission) on the air-water exchange in an oligotrophic north Patagonian lake of Chile. The monitoring was carried out in Panguipulli Lake (Los Ríos Region, Valdivia, Chile) during autumn-winter seasons (year 2017) due to higher possibility of PAHs emissions by incomplete combustion of organic materials released from surrounding areas. PAHs such as Methylnaphthalene (Mnap), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Fluo), Trimethylnaphthalene (Tnap), Phenanthrene (Phe), Anthracene (Ant), Methylphenanthrene (Mphe), Pyrene (Pyr), Chrysene (Chry), Benzo[b&k]fluoranthene (BbkF), Benzo[a]pyrene (BaP), Perylene (Per), Dibenz[a,h]anthracene (DBA), Coronene (Cor) were quantified in surface water and air matrices through GC-MS. To determine the freely dissolved concentration in water measured concentrations were corrected by dissolved organic carbon (DOC) for each sampling period. For understanding the short-term dynamic between water and air the fugacity ratios ($fw\,fa^{-1}$) for every day were calculated. Panguipulli Lake reported the occurrence of 15 PAHs in air (mean $\sum_{15}$PAHs= 11615 pg m^{-3}) and water (mean $\sum_{15}$PAHs= 961 pg L^{-1}), where the higher concentrations in the environment were quantified for Mphe (air_{max}: 8460 pg m^{-3}; $water_{max}$: 707 pg L^{-1}) and Pyr (air_{max}: 30533 pg m^{-3}; $water_{max}$: 2296 pg L^{-1}). Air-water diffusive exchanges of PAHs showed a tendency from a net volatilization ($\log fw\,fa^{-1} > 0.5$) for less hydrophobic ($\log K_{OW} < 4$) and lighter PAHs (i.e., Mnap, Acy, Ace, Fluo, Tnap) to net deposition ($\log fw\,fa^{-1} < -0.5$) for more hydrophobic ($\log K_{OW}$ 4 – 7) and heavier PAHs (i.e, Phe, Ant, Mphe, Pyr, Chry, BbkF, BaP, Per, DBA and Cor). A significant correlation was determined between log fugacity ratios and log K_{OW}s of PAHs (R-squared: 0.17; $p < 0.001$). This behavior of PAHs could suggest that the oligotrophic lake acts as a sink of organic pollutants derived from anthropogenic sources during autumn and winter seasons (pyrogenic source: Ant/Ant+Phe; petroleum and coal combustion: Fluo/Fluo+Pyr), being the heavier and more hydrophobic PAHs potential to reach bottom sediment. This study is a first approach to understand the source and diffusive transport of PAHs on oligotrophic lakes of the southern Chile. Acknowledgments to the FONDECYT-CONICYT for the projects awarded (postdoctoral-Fondecyt N° 3180159, initiation-Fondecyt N° 11150548, Regular-Fondecyt N° 1180063 and Fondap-Crhiam N°15130015).

MO038

Short term air-water exchange of Persistent Organic Pollutants (POPs) in the Concepción Bay, of Central Chile: Is the water body a source or sink of chemicals?

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Persistent Organic Pollutants (POPs) are organic compounds resistant to chemical and biological degradation and therefore they can persistent in the environment, they have the potential for bioaccumulation and then can be transferred through the food web until to reach humans. Coastal areas constitute key ecosystems because they provide a link between the ocean and human activities i.e., development of urban areas, industrial settlements, fisheries activities, and others. However, it is well known that the urban development associated with coastal areas lead to an increase in the levels of pollutants in the marine environment. Air and water samples were collected at two sampling sites (Tome and Talcahuano) in Concepción Bay in January 2014 during the Austral summer season. Polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) were analyzed by means of gas chromatography-mass spectrometry (GC-MS). Results showed POP concentrations in air and water were low, at the two sampling sites for PCBs (3 to 20 pg/m^3), for OCPs: 2 to 120 pg/m^3, and PBDEs (2 to 10 pg/m^3) in air; and in water for PCBs (5 to 40 pg/L), for OCPs: 5 to 40 pg/L, with exception of PBDEs (50 to 20000 pg/L) which was three order of magnitude higher than the other chemicals. Air–water exchange direction was estimated using the fugacity ratio between air and water, the results obtained showed that among the studied compounds HCH isomers and three PBDEs (47, 99 and 100) showed deposition, while on the other hand PCBs, PBDE 209 and HCB showed volatilization. Air-water exchange fluxes estimated ranged from 3.63 to 4736 ng m^2/d for PBDEs, from 1.44 to 23.2 for PCBs and from 1.1 to 32.6 for HCB. The estimated air-water exchange, at both sites, showed volatilization for most of the chemical analyzed with exception of HCHs which was close to the equilibrium state. This condition pointed out that water is the main source of POPs to the atmosphere during the Austral summer. In particular, the resuspension of sediments loaded with PBDEs residue from the Tsunami event that impacted Concepción Bay in February in 2010 due to a local upwelling event that affects the study area during summer.

MO039

Occurrence and Cycling of Persistent Organic Pollutant in an Oligrotrophic Patagonian Lake

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Persistent Organic Pollutants (POPs) are a diverse group of organic compounds, which are stable and resistant to degradation, prone to bioaccumulate in organisms and biomagnify through the food chain. As a semi-volatile product, they are subject to long-range atmospheric transport (LRAT) through the atmosphere or ocean currents. In addition, because of their toxicity levels, they are harmful to wildlife and human health. The final fate of POPs depends on their physicochemical properties and the processes of exchange between the different environmental compartments. The present work is the result of coupled sampling of air and water during autumn-winter seasons (year 2017). PCB 28, 52, 101, 118, 153, 158 and 180, α-HCB, HCB, β-HCH, ?-HCH, δ-HCH were analyzed using a GC-uECD. Average air and water concentrations for ∑PCB were 28,931 pg m-1 and 219,486 pg L-1; ∑HCB, 0,632 pg m-1, and 26.631 pg L-1; ∑HCH, 82,831 pg m-1, and 908.834 pg L-1, respectively, the concentrations were found to be among the lowest reported for the area both in air and water. For understanding the short-term dynamic between water and air the fugacity ratios ($fw\,fa^{-1}$) for every day were estimated using the concentrations corrected by DOC for each sample. The results showed that deposition dominates over volatilization in all analyzed PCB, HCH congeners, and HCB. The low concentrations found in the water could be explained due to the isolation of the study area and short residence times in the lake that affects the direction of exchange. To date, this is the first report of levels of Persistent Organic Pollutants in Lakes in South Chile.

MO040

Occurrence and distribution of persistent organic pollutants in tissues of green turtles (Chelonia mydas) from Australia

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Antonia Weltmeyer[1], Liesbeth Weijs[2], Gülsah Dogruer[2], Adrian Covaci[3], Henner Hollert[1] [1]Institute for Environmental Research (Biology V), RWTH Aachen University, Aachen, Germany [2]Australian Rivers Institute**,** Griffith University, Brisbane, Australia [3]Toxicological Center, University of Antwerp, Wilrijk-Antwerp, Belgium Persistent organic pollutants (POP) include various anthropogenic chemicals that pose different threats to biota due to their persistence, bioaccumulation and toxicity. They are released into the environment mostly as byproduct of industrial processes (e.g. PDCC/F), directly applied in agriculture (e.g. HCH, HCB) or distributed through incineration (e.g. PCB). While these contaminants have received considerable attention in some marine species, e.g. various marine mammal species, little is known about the bioaccumulation and partitioning of POPs in marine reptiles, such as the green turtle (*Chelonia mydas)*. Green turtles spend most of their life time in coastal areas like seagrass meadows and shallow waters where many anthropogenic chemicals are being released into the marine ecosystem. Even though biomagnification is comparatively low due to their mostly herbivorous lifestyle, POPs have been found in several green turtle populations around the world. Research has also shown comparatively higher POP concentrations in urban and rural agricultural areas of Australia. Such chemical pollution can lead to a general decrease of health and reproduction of the already endangered green turtle. In this study, different tissues (liver, kidney, muscle, adipose tissue) of green turtles from southeast Queensland, Australia, were investigated for their POP load. The following contaminants: 25 PCB congeners, 7 PBDEs, DDT and its major metabolite, DDE, 5 chlordanes, HCB, 3 HCHs and 2 naturally-produced methoxylated PBDEs were targeted. Bioaccumulation of POPs depends on several factors such as tissue type, log Kow values of the compounds, and the age of the animals. The influence of these factors on the partitioning and accumulation of the POPs was investigated in the turtles in this study. This study provides new data in order to raise awareness of the environmental pollution and its consequences for marine biota. Furthermore, an in-vitro bioassay for determination of dioxin-like activity (micro-EROD) will be conducted to assess the health impact of the determined POPs on an individual level.

MO041

Organic pollutants and trace elements as possible drivers for yeast

occurrence in lichens: a case study for an Antarctic ecosystem
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Antarctica, one of the least polluted regions on Earth, provides unique opportunities for studying environmental pollution processes at both local and global scales. The "global distillation" mechanism is majorly held responsible as the main, or even the only pollutant input process in Polar environments, whilst the biologically mediated transport is frequently not taken into account. However, the role of seabird colonies as relevant secondary sources of both Persistent Organic Pollutants (POPs) and Trace Elements (TEs) was recently reported in literature. The fact that these colonies represent the most important nutrients source to terrestrial ecosystems, inducing a pattern of vegetation around colonies where lichens normally occupy the drier and more distant spots, coupled to their role as pollutant sources results in comparatively high concentrations of POPs and TEs in lichens. In the present work, we hypothesised that these pollutants might influence the quantitative (number of isolates) and qualitative (taxonomic diversity) occurrence of yeasts in lichens from Maritime Antarctica ecosystems. The most common yeast species were *Vishniacozyma victoriae* and *Cystobasidium laryngis*, differently from the ones found directly on bird faeces, rejecting the hypothesis of colonization from the birds themselves. The number of isolates correlated significantly with the taxa and *C. laryngis*, meaning this species is more likely to appear in densely populated substrata. Negative significant correlations were found for the number of isolates with As and Cd, the same case being repeated for the taxa. Indeed another interesting result was revealed for taxa: a significant negative correlation to %N, likely due to a probable ecological dominance mechanism, but also due to the fact that %N and the levels of As showed significant positive correlation, which corroborates its toxicity to yeasts. Principal Components Analysis and Generalized Linear Models ranked by AICc suggested similar adverse influence considering POPs (PCBs and HCB). Therefore, our results suggested deletery effects regarding both yeast quantity and diversity linked to As, Cd, PCBs and HCB, all of them likely supplied by the colonies according to previous studies carried out by the authors. To the best of our knowledge, this is the first work to shed light on this issue.

MO042
Assessment of climate change influences on the exposure level of VOCs and PCDDs/DFs in soil and water of Seoul, Korea
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Influence of climate change (CC) was assessed on the environmental levels of three VOCs (benzene, toluene, and formaldehyde) and three PCDDs/DFs (2,3,7,8-TCDD, 1,2,3,6,7,8-HxCDF, and OCDF) using a regional-specific multimedia fate model (KPOP-CC). The predicted average concentrations in soil and water over each of the three 20 year periods (2021 to 2040 (F1), 2051 to 2070 (F2), and 2081 to 2100 (F3)) were compared to those of the period of 1986 to 2005 (BASE) under the RCP8.5 scenario in Seoul, Korea. The average concentrations of VOCs in soil and water increase by up to 109% and 87%, respectively, as compared to BASE. As CC progresses, however, the average concentrations gradually decrease to give F1>F2>F3. Model prediction indicates that the decreasing concentrations of VOCs in soil and water with the CC periods are governed by the increased volatilization loss to air followed by increased degradation. The average concentrations of PCDDs/DFs in bare soil and forest soil increase by up to 562% and 2300%, respectively, while by up to 419% in water. On a monthly bases, the increase in the concentration in water is over 3392% in June. Atmospheric PCDDs/DFs are input to soil mainly in the particulate form and accumulate in soil due to their persistence. In bare soil, PCDDs/DFs concentrations in F3 are lower than those in the periods of F1 and F2 because of the decrease in wet deposition of atmospheric particles in F3. In forest soil, however, wash-off of the particle-bound PCDDs/DFs from vegetation surfaces dominates during wet period, hence the average concentrations of PCDDs/DFs increase as CC progresses from F1 to F3. Increase of solid runoff caused by the increase in the precipitation intensity leads to the substantial increase of the concentrations in water. These results clearly demonstrate that i) the CC influence on the environmental level of chemical compounds can substantially vary with their physicochemical and fate properties and ii) CC may considerably increase the environmental health risk from neutral hydrophobic SVOCs (e.g., PCDDs/DFs) in the soil and water ecosystems.

MO043
Prediction of Bioaccumulation Potential for Ionizable Chemicals Based on COSMO-RS
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There is a high demand for reliable and robust methods to predict the bioaccumulation potential for agrochemicals, pharmaceuticals as well as industrial chemicals, in order to access the long-term and chronic toxicity. Currently, the bioaccumulation potential is assessed in a tiered approach via octanol-water partition coefficients at the first stage. However, this approach is not applicable for organic ions and ionizable chemicals, because the latter have different mechanisms of sorption. It is therefore important to develop new screening tools that work for ionizable and ionic chemicals.[1] As an alternative, sorption models are proposed including storage lipids, phospholipid membrane lipids and proteins. Partitioning into storage lipids (*rmsd* up to 0.35) and proteins (*rmsd* = 0.57), pK_a values and fractions of different protonation states are calculated by the COSMO-RS (Conductor-like Screening Model for Realistic Solvation) approach.[2,3,4,5] The COSMO*mic* extension for the COSMO-RS treatment of inhomogeneous phases is used to calculate the partitioning into biomembranes (*rmsd* = 0.70).[6] COSMO-RS is based on first-principle quantum chemical structures, and uses physically sound intermolecular interactions (electrostatic, hydrogen bond and van der Waals), including the handling of protonated states and ionic structures. For this reason, it is unbiased towards different application scenarios, as industrial chemicals, agrochemicals and pharmaceuticals. Two approaches will be demonstrated: at first, a static model, based on a recently published model for the bioconcentration potential in fish[1]; at second, a simple toxicokinetic model for terrestrial organisms, which does take into account the uptake via different routes and biomembrane permeabilities. The latter could allow for a species-to-species extrapolation and, thus, a consideration of impacts on non-target species in the ecosystem. [1] Bittermann et al., Environ. Sci.: Processes Impacts, 2018, 20, 845 [2] Klamt et al., J. Phys. Chem. A, 1998, 102, 5074; [3] Klamt, WIREs Comput. Mol. Sci., 2017, e1338 [4] Klamt et al. J. Comp. Aid. Mol. Des., 2016, 30, 959 [5] Geisler et al., Environ. Sci. Technol. 2015, 49, 5538 [6] Bittermann et al., J. Phys. Chem. B, 2014, 14833

Endocrine Disruption in Invertebrates: Historical Perspectives, New Developments, and Key Research Needs (P)

MO044
Acute toxicity of ethylparaben to Daphnia similis
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The increasing industrial activity along urban growth has accelareted the disposal of several pollutants in water bodies. Analytical methods have been developed as there is an arising concern with emerging contaminants. These chemical substances are found in surface water in low concentrations and can resist to the sewage and industrial wastewater treatment systems. Parabens are considered a noxious group of chemical preservatives with high industrial usage that threaten both the human health and the biologic communities of aquatic ecossystems due to its toxicity and their endocrine disruption potential. Ethylparaben, one of the most widely used variety of the parabens can also be accumulated throughout the trophic levels because of its high k_{ow} value. Therefore, it is extremely important to evaluate its toxicity for environmental legislation and ecological risk assessment. Cladocera represents one of the most important group in the zooplanktonic community regarding energy transfer along the food chain. Because of their short life cycle and their high sensibility to aquatic pollutants, they are widely used in ecotoxicological tests. This study evaluated the sensitivity of *Daphnia similis* to ethylparaben according to the OECD guideline 202 on *Daphnia* sp., acute immobilization test and reproduction test. The tests concentrations were determined by HPLC-DAD analyses. Comparing the results of the present study to the literature, it may be concluded that the *Daphnia similis* specie (EC50 = 23.70 mg L-1) is more sensitive to ethylparaben than the *Daphnia magna* (EC50 = 32 mg L-1), the *Dugesia japônica*(EC50 = 31 mg L-1) and the *Photobacterium leiognathi*(EC50 = 25 mg L-1). Many studies of fresh water monitoring have reported the presence of parabens in concentrations of μg L-1. This emphasizes the importance of researching the effect of environmental concentrations of parabens on reproductive, fisiologic and comportamental parameters of *D. similis* specie.

MO045
Adverse effects of mono-(2-ethylhexyl)-phthalate on Daphnia magna in acute and chronic test
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Mono-(2-ethylhexyl)-phthalate (MEHP) is the metabolite of di-(2-ethylhexyl)-phthalate (DEHP), which is widely used in the industry as plasticizers. According to previous studies, both MEHP and DEHP causes oxidative stress and are considered as reproductive toxicants in various organisms. MEHP is highly persistent and bioaccumulative in environment and living organisms. However, little is known about toxicity of MEHP to aquatic invertebrates such as *Daphnia magna*. The aim of this study was to elucidate the mechanism of MEHP toxicity in *D. magna*. It is interesting that LC_{50} of MEHP were 85.2 mg/ L^{-1} for 48 h which

were higher than known LC_{50} (0.56 mg L^{-1}) of DEHP. We studied the effects of oxidative stress as molecular initiating events on *D. magna*. *D. magna* (5 d) were exposed to MEHP for 6 h and 24 h for observing the changes in different levels of the Reactive Oxygen Species (ROS), glutathione S-transferases (GSTs), GSH/GSSG ratio. In addition, expression levels of glutathione related genes and juvenile hormone genes were utilized to understand the toxic mechanism of MEHP. Lastly, the reproduction rate and growth of daphnids during chronic (21 d) test were included in this study. Further study is needed to clarify how MEHP leads to dysfunction of reproduction system of *Daphnia magna*.

MO046

Biomarkers of intersexuality and reproductive dysfunction in crustaceans

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Despite the well-documented cases of endocrine disruption in vertebrates and some molluscan groups, it is currently unknown whether reproductive endocrine disruption represents a threat to crustacean populations. The study of widespread intersexuality provides an opportunity to investigate sexual differentiation and dysfunction. Crustacean intersexuality is associated with contamination and includes forms linked to increased sex-ratio distorting parasites at polluted sites. Despite the importance of crustaceans for monitoring vulnerable aquatic habitats, little is known about the molecular basis intersexuality or any associated sexual dysfunction. To increase the relevance of crustaceans to environmental toxicologists, this study comprehensively analyzed gene expression in amphipods presenting parasite and non-parasite associated intersexuality. We suggest existing vertebrate biomarkers of feminization should not be applied to crustaceans, as orthologous genes are not induced in feminized amphipods. Furthermore, in contrast to vertebrates, where feminization and intersexuality are often associated with deleterious de-masculinization, we find males maintain masculinity even when unambiguously feminized. This reveals a considerable regulatory separation of the gene pathways responsible for male and female characteristics and demonstrates that evidence of feminization (even if detected with appropriate biomarkers) is not a proxy for de-masculinization in crustaceans. This study has also produced a comprehensive spectrum of potential molecular biomarkers that are currently being applied to specimens collected from clean and industrially impacted locations. This will represent the first application of appropriate transcriptomic biomarkers to monitor feminization and de-masculinization in field populations of crustaceans.

MO047

Characterization and transcriptional response of ecdysone receptor gene in the mud crab Macrophthalmus japonicus: effects of osmotic stress and endocrine disrupting chemicals

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Ecdysone receptor (EcR) induces hormonal pathways by binding ecdysteroids to other nuclear receptors for development, reproduction, and molting processes. To further understand the role of EcR in polluted environments, we have characterized EcR, and assessed transcriptional responses of the EcR gene to osmotic stress, and to endocrine disrupting chemicals (EDCs) such as bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP) in the intertidal mud crab *Macrophthalmus japonicus*. Phylogenetic analysis showed that the ligand binding domain of *M. japonicus* EcR formed two different clusters among crab species. Osmotic stress prompted different expression patterns of EcR mRNA in *M. japonicus* crabs under low and high salinity. BPA exposure induced significant up-regulation of EcR as early as day 1 in *M. japonicus* gills and hepatopancreas. However, the expression levels of EcR returned to similar, or lower, levels as controls, by days 4 and 7. In contrast, EcR mRNA expression was significantly induced in *M. japonicus* gills and hepatopancreas exposed to all concentrations of DEHP over all exposure times. Hepatopancreatic tissue is more sensitive than gill tissue in terms of EcR gene responses to EDCs. Taken together, these results suggest that the role of EcR in endocrine protection involves different processes, according to the type of EDCs involved, and that up-regulatory responses by EcR indicate the response of hormonal regulatory processes to environmental changes induced by osmotic stress or EDC exposure.

MO048

Chronic effects of bisphenol S and bisphenol SIP on Daphnia magna and Moina macrocopa and ecological risk assessment

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In response to the regulatory pressures to eliminate bisphenol A (BPA) in plastics, bisphenol S (BPS) and 4-hydroxyphenyl 4-isoprooxyphenylsulfone (BPSIP) have been frequently used in manufacture of thermal paper and plastic containers. However, there is paucity of information on their chronic toxicity using aquatic invertebrates and associated ecological risk. In the present study, chronic toxicity of BPS and BPSIP were evaluated using two freshwater invertebrate species, i.e., *Daphnia magna* and *Moina macrocopa*. The body length was significantly decreased in *D. magna* exposed to 5 and 10 mg/L of BPS, and 1 mg/L of BPSIP. The reproduction NOEC value in *M. macrocopa* was 0.03 mg/L of BPS and 0.1 mg/L of BPSIP. Based on the toxicity values from the present study and literature, the predicted no effect concentration (PNEC) of BPA, BPS, and BPSIP was calculated. For the BPA and BPS in which the measured environmental concentration of surface water worldwide is present, the hazard quotient (HQ) was derived for the ecological risk assessment. The HQs of BPA and BPS toward aquatic organisms were calculated to be greater than 1.0, indicating that potential ecological risk currently. With increasing use of these alternative compounds, more monitoring program in aquatic environment and study of toxicity mechanism appeared to be necessary. Acknowledgement - This study was supported by the National Research Foundation of Korea (Project NRF-2015R1D1A1A01056628).

MO049

Comparative study of the effect of bisphenol A (BPA) and two analogs, bisphenol S (BPS) and bisphenol F (BPF), on the endocrine system of Chironomus riparius larvae

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There is increasing evidence that bisphenol A (BPA, 2, 2-bis [4-hydroxyphenyl] propane), used in plastics and other products, could be harmful to human health due to its association with diseases such as obesity, diabetes, reproductive disorders and cancer. Many *in vitro* and *in vivo* research studies, have demonstrated the ability of BPA to act as an endocrine-disrupting chemical (EDC) and have attributed its exposure to cytotoxic, genotoxic and carcinogenic effects. BPA has been widely used for the manufacture of polycarbonate plastics and epoxy resins. The exposure of humans occurs through food in contact with the materials that contain them, such as polycarbonate bottles, plastic food containers, etc. So, bisphenol S (BPS, bis [4-hydroxyphenyl] sulfone) and bisphenol F (BPF, bis [4-hydroxyphenyl] methane) are already being used for a variety of industrial applications as BPA alternatives. BPS is used as an agent in cleaning products and as a developer in thermal paper, including products marketed as "BPA-free paper". BPF is used to make epoxy resins, coatings, adhesives for several consumer products, food packaging, etc. BPA-like effects of BPS and BPF can be hypothesized because their chemical structures are similar. Some studies have analyzed *in vitro* the hormonal actions of BPA analogues, demonstrating they have actions similar to those of the original compound, which supports the possibility of being hormonally active. However, it is necessary to study the physiological effects and endocrine activities of BPA substitutes *in vivo*. The main objective of this study was to evaluate different metabolic pathways of action of BPS and BPF compared to BPA, in four instar *C. riparius* larvae, an aquatic invertebrate widely used in environmental toxicology. The transcriptional activity of four genes involved in the endocrine pathway (Cyp18a1, shadow, ECR and E74), one gene related to apoptosis (DRONC) and a multi drug resistance gene (MRP1) were evaluated by real time RT-PCR in *C. riparius* larvae during 24 hours of BPA, BPS and BPF exposures at 0.05, 0.5 and 1 mg/L. The obtained results show similar patterns of BPA, BPS and BPF transcriptional expression when compare the Cyp18a1, DRONC, and shadow genes. These results suggest a similar gene activation model for the three compounds employed, which implies the need to carry out more *in vivo* studies to evaluate the hormonal actions of BPA analogues. Possibly BPF and BPS are not a safe alternative to BPA.

MO050

Effect of glyphosate-based herbicides on relative transcript expression levels of genes involved in growth and reproduction in the decapod Macrobrachium potiuna

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Glyphosate based herbicides (GBH) are the most commonly used herbicides worldwide. In the environment, glyphosate has been detected in sediments, surface and groundwater. Thus, the species that inhabit these ecosystems are exposed to this contaminant. Although, animals lacks the metabolic pathway of the GBH action, several studies has been demonstrating the toxic effects of GBH exposure on animals, mainly related to oxidative stress, genotoxicity, cytotoxicity, neurotoxicity and teratogenicity. Moreover, for vertebrate models, glyphosate is known as an endocrine disruptor, mimicking the effect of sexual and growth hormones, thus, biomarkers for the assessment of endocrine disruption caused by GBH exposure have been developed. However, currently, no biomarkers are available to assess the endocrine disruption in decapod crustaceans exposed to GBH. Thus, the aim of this study was to assess the effects of low GBH concentrations on the relative transcript expression levels of the molt-inhibiting hormone (MIH), the ecdysteroid receptor (EcR) and the vitellogenin (Vg) genes involved in the process of molting and reproduction on the prawn *Macrobrachium potiuna*. Males and females were exposed to GBH (0.00; 0.0065, 0.065 and 0.28 mg L^{-1}) for 7 and 14 days. Next, the relative transcript expression levels of these three genes have been measured by RT-qPCR, for each gender and time of exposure. Males exposed for 7 days highlighted overexpression of EcR and MIH, while the opposite was observed in Vg, being overexpressed in prawns exposed for 14 days. Furthermore, it was observed a time-dependent effect mainly in the two higher concentrations. For females exposed for 7 and 14 days, no alteration was observed in EcR and Vg expression, but it was observed a time-dependence effect mainly in the 0.0065 and 0.065 mg L^{-1} concentrations. Therefore, this work underlined that exposure to GBH also affect invertebrate species and can modulate the expression of genes involved in the molting and reproduction. The obtain results are promising in the development of future biomarkers for GBH exposure in crustaceans. Nonetheless, further studies are needed to better understand the variations of these genes and their application in field assays, before to use them as biomarkers.

MO051

Effects of anti-androgen compounds on the relative expression of the retinoid X receptor gene of the rotifer Brachionus calyciflorus

E. Gismondi, LEAE - University of Liege / Laboratory of Animal Ecology et Ecotoxicology; L. Woeffler, University of Liege; C. Joaquim-Justo, University of Liege / Laboratory of Animal Ecology et Ecotoxicology

Rotifers are widely used as bio-indicators and models for ecotoxicology due to their sensitivity to various toxicants, but also other characteristics such as short generation time, ease of culture in small volumes, ease of establishing clone cultures. Although many studies highlighted that endocrine disruptor compounds (EDCs), especially anti-androgen compounds, impact the population fitness of rotifers (e.g. population growth, fecundity, cyst production), endocrine mechanisms mediating these effects remain obscure, probably due to the limited knowledge on endocrine systems in rotifers. The retinoid X receptor (RXR) is a nuclear receptor which has been shown to be involved in the imposex induction in gastropods. Based on this information, we aimed to evaluate in the monogonont rotifer *B. calyciflorus*, the effects of three anti-androgen compounds (i.e. flutamide, fenitrothion, cyproterone acetate) on the relative expression of the RXR gene, in order to improve the understanding of the mechanisms of action of these EDCs. For each compound, three concentrations were chosen according to their respective NOEC value, and *B. calyciflorus* were exposed for 6hrs, 12hrs and 24 hrs. Results highlighted that RXR relative expression was under-expressed in *B. calyciflorus* exposure at the three anti-androgen compounds, suggesting that RXR could be involved to demographic effect previously observed (e.g. decrease in resting eggs produced, fertilization – Tian et al. 2017). Nevertheless, more investigations are needed to understand the endocrine disruption mechanism.

MO052

Effects of tributyltin (TBT) exposure on gametogenesis and sex ratio in the European flat oyster (Ostrea edulis)

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Tributyltin (TBT) compounds have been used for various purposes including antifouling paints for boats, preservatives and antimicrobial agents. Although restricted in many countries due to their relative persistence, tendency to bioaccumulate and toxicity, they are now widespread global contaminants that persist in natural environments. TBT, and its derivatives, have shown adverse effects including mortality and masculinization in form of imposex or intersex in gastropods. Although TBT has been one of the most extensively studied examples of endocrine disruption in gastropods, the underlying mechanism in oysters remains unclear. The flat oyster *Ostrea edulis* (Linnaeus, 1758) is a commercially valuable species in Europe but its populations have collapsed in recent decades. In the Solent, Southern England, one such populations of oysters has seen a significant reduction in the number of brooding female-phase oysters and skewed sex ratio towards male-phase oysters in recent years. In order to understand the effect of TBT exposure on gametogenesis and the potential mechanism of disruption on gender determination, oysters (n=30) were exposed to 20 ng/L, 200 ng/L and 2000 ng/L TBT chloridefor two months. At the end of the exposure, gametogenic stage and sex ratio were assessed histologically and metabolomic profiling analysis was conducted to elucidate themetabolic alterations that occur in individuals exposed to TBT. Our results showed a significant concentration-dependent increase in mortality. Moreover, at 20 ng/L and 200 ng/L TBTCl the proportion of *O. edulis* individuals developing as males or were undifferentiated increased respect to the control group. At 2000 ng/L TBTCl no males were found but the proportion of undifferentiated oysters was significantly higher compared to the control. The metabolome of TBTCL exposed oysters demonstrated changes in energy metabolism-related pathways and cortisol levels as compared to control group. In total, our results indicate that gametogenesis and sex determination in *O. edulis* can be affected by TBT exposure creating a bias toward males this species. However, high concentrations of this compound could increase mortality rather than effect reproduction. The presence and persistence of TBT in the environment could cause an additional threat to the declining *O. edulis* populations around the world, by increasing mortality, changing reproductive maturation and skewing sex-ratios in natural populations.

MO053

Endocrine disruptors in the aquatic environment: Is it enough to screen for estrogens only?

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Endocrine disrupting chemicals (EDCs) mimic the behaviour of natural hormones and are suspected to disturb reproduction and development in wildlife (1, 2). The chemicals receiving most attention are those interfering with functions of the vertebrate sex hormone estrogen, the so-called xenoestrogens (2, 3, 4). Potential EDCs that may affect other ecologically and economically relevant phyla that do not possess estrogen receptors, such as aquatic arthropods (e.g. daphnids, shrimps and crabs) are rarely addressed (5). The key hormone in arthropod molting is 20-hydroxyecdysone (20E), yet the response of the ecdysone receptor (EcR) to environmental stressors (e.g. insecticides with endocrine disrupting potential) is not well understood (6, 7). Since the EcR system is highly conserved across taxa, non-target organisms of insecticides, such as crustaceans, could be adversely affected (5). Bisacylhydrazines, a widely applied group of insecticides in fruit farming, act as non-steroidal 20E agonists that can disrupt normal EcR function (3, 6, 8). They are supposed to be highly species-specific and thus environmentally safe (3, 9). This, however, is contradicted by reports of toxicity in the low concentration range of $\mu g\ L^{-1}$ of active ingredient towards non-target organisms, e.g. the crustacean *Daphnia magna* (9, 10). Two receptor activity luciferase reporter gene assays (11, 12) will compare the effect of environmental samples on estrogen and ecdysone receptor activities. Sampling stations cover different pollutant scenarios. They are located in proximity to a waste water treatment plant, where higher levels of estrogenic compounds are expected, in addition to areas of extensive fruit farming where ecdysonic compounds might prevail. 1. Berckmans. et al. (2007) *Chemosphere* 21: 1262–1267, 2. Schmitt et al. (2012) *Environ Sci Pollut Res 1*9: 3350–3361, 3. Hahn et al. (2001) *Ecotoxicol Environ Saf* 49(2): 171–178, 4. Vethaak et al. (2017) *Mar Environ Res* 124: 81-91, 5. De Wilde et al., (2013) *Aquat Toxicol*, 130–131: 31–40, 6. Song et al. (2017)*Environ Si. Techn* 51(8): 4142–4157, 7. Nakagawa & Henrich (2009) *FEBS Journal* 276(21): 6128–6157, 8. Dhadialla et al. (1999) *Ann Rev Entomol*, 43(1): 545–569, 9. EEDB (1995) US EPA, Washington DC, 10. Pest Management Regulatory Agency (1996) PRDD96-01, 11. Legler et al., (1999) *Toxicol Sci* 48: 55-66, 12. Song, et al. (2017) *J Toxicol Environ Hea-Part A Curr. Issues:* 80(16–18), 954–962

MO054

Transcriptional profiling of heat shock proteins expressions to osmotic and endocrine disrupting stress in mud crab Macrophthalmus japonicus

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Heat shock proteins (HSPs) play crucial roles in the cellular metabolite process as well as protective immune responses.In the study, we investigated the transcriptional profiling of six HSPs in mud crab *Macrophthalmus japonicus* under osmotic and endocrine disrupting chemical stress. The mRNA expressions of HSPs showed differently in the gills as up-regulations of HSP70, HSP90, and HSP40 or down-regulations of HSP60 under high salinity. In hepatopancreas, under osmotic stress, the responses of most HSPs genes showed up-regulation at early exposure time and down-regulation at late exposure time. In addition, Bisphenol A (BPA) exposure mostly induced responses of HSPs in gills. In hepatopancreas, HSP70 and HSP90 gene expressions showed up-regulation, whereas the transcriptional expressions of HSP60, HSP83, and HSP21 showed down-regulation at day 1 and day 4. These results suggest that HSPs are involved differently in the molecular adaptation and defense responses of crabs to BPA and osmotic stress.

MO055

Using a RT-PCR array for the analysis of single, binary, and ternary mixture exposure of UV filters Octocrylene and OD-PABA and Bisphenol A on Chironomus riparius.

A.M. González, UNED / Mathematical Physics and Fluids; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos

The presence of UV filters in the environment has been increased in recent years, cataloging them as emerging pollutants. The aquatic systems are especially affected due to recreational activities as well as the absence of specific treatments in Waste Water Treatment Plants (WWTP). Previous studies showed endocrine disruption, but studies focused on invertebrate organisms are scarce. In parallel, bisphenol A is one of the main plasticizers used in packaging and other industrial products. Multiple studies have evaluated its effects, concluding that it is an endocrine disruptor. In order to make an evaluation closer to the environmental conditions, we have analyzed the effects of individual, binary, and ternary mixtures of Bisphenol A and two UV filters, Octocrylene (OC) and 2-ethylhexyl 4- dimethylaminobenzoate (OD-PABA), on fourth instar larvae of *Chironomus riparius*. These mixtures usually reach to freshwater ecosystem so the main objective was to determine the effects in gene expression of this species, which has a relevant role in the food chain of these ecosystems. In order to evaluate the response at short and medium term, larvae were exposed for 24 and 96 hours to 0.1 and 1 mg/L of single compounds and to binary and ternary mixtures. It is known that exposure to toxicants can alter different metabolic pathways, generating a domino effect. Thereby, the expression profile of 40 genes was analyzed by retrotranscription and Real-Time PCR using a specific array covering a number of relevant metabolic pathways related with endocrine system, immune response, stress response, detoxification mechanisms, and apoptosis among others. These studies open up new opportunities in the design and identification of molecular biomarkers for toxicological evaluation and ecological risk assessment. The methodology used in the design of the array can be extrapolated for other species. This work has been funded by the Ministerio de Economía y Competitividad, CICYT (SPAIN), CTM2015- 64913-R. A.B.M.G is the receiver of a pre-doctoral contract from the National University of Distance Education (UNED).

MO056

Validation of the protocol of juvenile hormone activity screening assay (JHASA) proposed to OECD

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After we had found the juvenile hormone agonists for *Daphnia magna* more than ten years ago by Tatarazako et al. (Chemosphere, 53:827-33, 2003), we have found several agonists such as diofenolan (Abe et al., Aquat Toxicol, 159:44-51, 2015). We had also proposed a confirmation test protocol for juvenile hormone to OECD and it was admitted as annex 7 of Guideline for Testing of Chemicals No. 211. However, it takes whole 21 days of the testing period to fully conduct this standardized test and it is highly time-consuming to observe all the neonates to check their sex. So, we proposed short-term screening test using *D. magna* of 10-17 days old based on our previous study (Abe et al., J App Toxicol, 35:75-82, 2015) to observe the sex of the neonates for seven days, and currently under discussion in OECD expert meeting as Juvenile Hormone Activity Screening Assay (JHASA). The experts also commented to concern the possible effects of non-chemical stress such as temperature, light cycle, and hardness. So, we decided to conduct ring tests with five laboratories (three CROs, a chemical manufacturer, and our institute) in Japan to validate the proposed protocol of JHASA using two chemicals, one is a positive control and the other is a negative control. The results were compared in detail in terms of the difference in the laboratories and slight difference in *D. magna* strain. We are also going to show some of the preliminary results of the potential non-chemical stresses.

MO057

Effects of ten bisphenol analogs on thyroid endocrine system and possible interaction with 17β-estradiol using GH3 cells

J. Lee, Yongin University; S. Kim, Seoul National University; K. Choi, Seoul National University / School of Public Health; K. Ji, Yongin University

In response to the bisphenol A (BPA) regulation, various bisphenol analogs (BPs) have been widely used. However, the potentials of thyroid endocrine disruption and mixture toxicity with other environmental contaminants were not investigated as much as their estrogen disruption potential. In the present study, two sets of *in vitro* rat pituitary (GH3) cell assays were designed. In the first set of experiments, changes in GH3 cell proliferation were examined after 48 h and 96 h exposure to each of ten BPs (BPA, BPAF, BPAP, BPB, BPC, BPF, BPM, BPP, BPS, BPZ), in the absence or presence of a median effective concentration (6.4×10^{-10} M) of triiodothyronine (T3). All tested BPs maximally stimulate GH3 proliferation at a dose of 10^{-6} M, suggesting that BPA and its analogs behave like thyroid hormone (TH) agonist, at least some extent. BPs did not potentiate the T3-induced cell proliferation at 48 h exposure, while several tested BPs including BPA, BPAF, BPB, BPF, BPS, and BPZ elicited a potentiating effect on the T3-induced cell proliferation at 96 h exposure. These results indicate that TH-antagonistic effects of BPs depend on the tested dose and exposure time. In the second set of experiments, one of the most potent BPs, i.e., BPAF, was selected, and its possible interaction with 17β-estradiol (E2) on the thyroid endocrine system was evaluated. A combined treatment with BPs and E2 induced additive-like effects on GH3 cells, and the increase in cell proliferation and inhibition of gene transcription (*Trα*, *Trβ*, and *Dio2*) were more pronounced with the BPAF and E2 combination than with the BPA and E2 combination. Our observations suggest that adverse effects might be more pronounced using BPA analogs, and the effects of BPA on the thyroid endocrine system might be even greater than that expected, because of potential interactions with other common hormones such as E2. Although detailed mechanisms and implication of endocrine disruption by BPs warrant further studies, our findings are important as E2 is naturally present in biota and therefore BP absorption by organisms would inevitably lead to an interaction with E2.

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MO058

SETAC Endocrine Disruptor Testing and Risk Assessment Interest Group

L.A. Baumann, University of Heidelberg / Aquatic Ecology and Toxicology

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Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Exploring New And Novel Approaches (P)

MO059

Development of Short-Term Toxicity Test Methods to Estimate Chronic Toxicity using the Freshwater Parthenogenic Mayfly, Neocloeon triangulifer

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For decades, toxicity tests with aquatic invertebrates have been conducted and yet a small number of model organisms are routinely used. Test organisms are usually easily cultured in the laboratory, have rapid life-cycles, exhibit sensitivity to a variety of pollutants with reproducible results, and are generally available year-round. The US EPA effluent testing program uses short-term chronic freshwater tests (4d to 8d) with cladocerans (Cladocera, *Ceriodaphnia dubia)*, green algae (Sphaeropleales, *Raphidocelis subcapitata*) and fish (Cypriniformes, *Pimephales promelas)*. These species have been used extensively in acute and short-term toxicity tests using EPA standardized methods to assess the hazard of chemicals and effluents in freshwater environments. EPA has standardized *Hyalella azteca* (Amphipoda) and *Chironomus dilutus* (Dipteran*) test*methods for sediments; yet EPAs effluent and ambient testing manuals don't provide acute or short-term test methods for *H. azteca*, *C. dilutus* or mayflies (Ephemeroptera, *Hexagenia*, *Neocloeon*). To add a sensitive insect, we have focused on using the mayfly, *N. triangulifer*, as it is parthenogenetic, has a short life cycle (~30d at 25C), and is sensitive to various toxicants. While methods for conducting acute 4d and chronic (~25-30d) toxicity tests with this mayfly have been published, a need exists to extend and standardize the methodology for applicable methods for testing in short-term exposures (e.g., 7d or 10d). Studies began with identifying an optimal starting age, test duration, and optimal sublethal endpoint for whole effluent toxicity testing. While others have compared the sensitivity of this species at 0d, 3d, & 5d old, we sought to further investigate this question with independent experiments comparing 0d and 7d old organisms in 7d and 14d tests. We developed a length versus dry weight relationship for this mayfly; dry weight is a more sensitive endpoint than length, but length is easier to measure more consistently and accurately with young instars. Efforts to refine the various aspects of diatom culture technique on food quality and therefore mayfly growth are underway and optimizing the diet for these organisms may be critical for achieving consistently high growth rates with low intra-treatment variability. Results of the study should provide data needed to guide the development of a toxicity test method to support NPDES permit decision-making. *This abstract does not necessarily represent the position or policy of the USEPA*. \n

MO060

Development of Short-Term Toxicity Test Methods to Estimate Chronic Toxicity using the Freshwater Mussel (Fatmucket, Lampsilis siliquoidea)

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For decades, toxicity tests with aquatic invertebrates have been conducted, and yet a small number of model organisms are routinely used. The US EPA effluent testing program uses short-term chronic freshwater tests (4 to 8d) with cladocerans (Cladocera, *Ceriodaphnia dubia)*, green algae (Sphaeropleales, *Raphidocelis subcapitata*) and fish (Cypriniformes, *Pimephales promelas)*. These species have been used extensively in acute and short-term toxicity tests using USEPA standardized methods to assess the hazard of chemicals and effluents in freshwater environments. While USEPA has standardized *Hyalella azteca* (Amphipoda) and *Chironomus dilutus* (Dipteran*)* test methods for sediments; USEPAs effluent and ambient test manuals don't provide acute or short-term test methods for *H. azteca*, *C. dilutus*, mayflies or mussels. To add another sensitive species, we have focused on method development using the fatmucket mussel (*Lampsilis siliquoidea*) for effluent toxicity assessments. A feeding study was conducted using a mixture of algal and shellfish foods with various starting ages of juveniles (~1, 2, 3 wk old mussels) in both 7d and 10d tests. Growth (shell length) at the feeding rate of 2 mL/2X/d with either 1 or 2 wk old mussels (~0.3-0.4 mm) and 3 mL/2x/d for 3 wk old mussels (~0.5 mm) provided consistent growth. Mean survival was ≥93% in all feeding treatments except for the 4 ml/1x/d to 1 wk-old mussels (78%). The increase of mussel shell length ranged from 24-52% at 7-d and from 28-60% at 10-d among four feeding treatments. Once the optimum feeding rate was determined, three ages of juveniles were exposed to a reference toxicant (NaCl) for 7d & 10d tests. For both durations, growth (length) was consistently more sensitive than survival, and with juvenile mussels growing over 50% in a short-term test (7d or 10d). The sensitivity to NaCl was mostly the same when tests were initiated with 1, 2 or 3-week-old mussels. To further evaluate the performance and variability in the mussel method, an interlaboratory study with 13 volunteer laboratories from the United States and Canada was conducted using 1-wk-old mussels and NaCl for a 7-d test. In this poster, we will present detailed results of the feeding studies, the relative sensitivity and the interlaboratory results along with a discussion of how the mussel procedure can complement current effluent and receiving water testing. *This abstract does not necessarily reflect the views or policies of the US Environmental Protection Agency. \n*

MO061

A new network of in silico models to cope with REACH requirements for (Q)SAR and read-across: the LIFE CONCERT REACH project

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LIFE CONCERT REACH is a new EU funded four-year project started in September 2018 [1] in which research institutes and consultancy companies are involved. The project is dedicated to establish a network of *in silico* models (i.e. (Q)SAR and automated read-across). On the one hand, it aims to link existent and well established *in silico* platforms (i.e. VEGA, The Danish QSAR database and AMBIT) to a new “super” platform. On the other hand, it will integrate new models, with the aim of covering virtually every possible environmental and (eco)toxicological endpoint required within the framework under Regulation EC (No) 1907/2006 (REACH). The development and implementation of an automated system is one key objective of the project, aiming to integrate several new *in silico* approaches, and to develop an automated tool able to combine multiple results (including potentially available experimental data) using weight of evidence approaches (WoE). The ambitious goal of the project is to offer more than 300 freely available *in silico* models within the same platform. Both (Q)SAR models and read-across tools will be developed or updated to be compliant with current regulatory requirements under REACH (e.g. ECHA’s Practical guide 5 - “How to use and report (Q)SARs” and ECHA´s “Read-across assessment framework (RAAF)”), focusing on providing all the information needed to evaluate and use the obtained results (e.g. QMRF and QPRF). The organisation of international workshops as well as targeted webinars for stakeholders (e.g. regulators and representatives of chemical industries) will establish a productive stream of information between the potential end users and the beneficiaries involved in the project, improving the usability and relevance of the newly developed software solution. **References** [1] LIFE CONCERT REACH - Concerting experimental data and in silico models for REACH; project reference LIFE17 GIE/IT/000461; http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=6791. Accessed 23 November 2018

MO062

Alternative approaches: how Firmenich has used the 3Rs to reduce the number of fish for REACH submissions

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Firmenich registered ~100 substances as lead (or technical) registrant, with more than 1/2 of the substances in tonnage band 1 (1-10 tpa), 1/4 in tonnage band 2 (10-100tpa), 1/10 in tonnage band 2 (10-100tpa), and one substance in tonnage band 4 (>100tpa). Around 1/2 of the substances were mono-constituents, 1/3 multi-constituents and 1/4 UVCBs. To complete the information requirements, almost 2000 data points were required of which 1/3 were fulfilled using experimental values (studies conducted) and the rest with alternative approaches, i.e. waiving applying the relevant specific (Column 2 of Annexes VII to X), or general rules as stipulated in Annex XI. When applying general rules, (Q)SARs, *in vitro* methods, weight of evidence and grouping approach were considered, following the relevant ECHA Guidance and criteria for acceptance. In order to fulfill the information requirements for fish acute toxicity, 31 fish acute toxicity tests were conducted (OECD 203), while in 15 cases alternative approaches (mainly (Q)SARs) were used. ECOSAR as well as ISafeRat® models were used alone or in combination. On 2 occasions (Q)SARs were used to predict the fish acute toxicity of the transformation products. Regarding fish chronic toxicity, one early-life stage test (OECD 210) was completed in 2003 and the OECD 212 was used as alternative for technical reasons to derive a surrogate EC10, while this endpoint was waived in 10 cases and in one case the NOEC was predicted using iSafeRat®. No fish bioconcentration test (OECD 305) was conducted for the purposes of REACH dossiers, however available fish bioconcentration data or predictions were submitted in 30 cases. 1/3 of the data were existing bioconcentration tests results, while the remaining data gaps were filled with BCF predictions, when it was felt that this was appropriate. Various models were used, alone or in combination (BCF/BAFWIN, OASIS BCF Baseline model). QSARs were also used, mostly as WoE to waive and support existing studies (especially when fish data were required; When generating new information, and also to determine WAFs Lethal loading rates for mixtures to determine worst case toxicity using the iSafeRat® model as animal tests were performed as a last resort. Overall, it can be concluded that by applying alternative approaches for fish tests, we were able to reduce the use of fish by ~5000, while maintaining a high level of confidence in the hazard and safety assessments.

MO063

An MOA-consensus classification in the EnviroTox database

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In the last decade, several mode of action (MOA) frameworks have been developed in aquatic ecotoxicology, mainly based on fish toxicity or associated with acute fish toxicity predictions. They provide information on a key determinant of chemical toxicity, but definitions of MOA used are unique to each of the classification schemes, therefore they lack harmonization and can give contradictory results. As a result, although the existing MOA frameworks have great potential to improve risk assessment and strengthen the use of alternative methods, a single reliable tool for MOA assignment still eludes ecotoxicology. The EnviroTox database contains curated *in vivo* aquatic toxicity results relevant for ecological risk assessments and contains a total of 91,217 records representing 1,563 species, and 4,016 chemicals. The database also includes information such as chemicals’ physico-chemical properties or MOA classification according to the Verhaar (modifed) framework, ASTER, USEPA TEST, and LMC OASIS fish toxicity models. Additionally, USEPA New Chemical and ECOSAR chemical category information has been added to provide information on structure-based grouping potential. The objective of this study was to critically evaluate this dataset with the aim of developing a ‘consensus’ MOA assignment for each chemical. A set of consensus rules was proposed based on a four model MOA comparison undertaken for the substances in the EnviroTox Database. A consensus was sought so that binary decisions could be made to classify those chemicals as non-specifically acting (i.e., narcotic), specifically acting or non-classifiable. In addition, a confidence rank was assigned to each MOA designation based on the degree of model consensus. This provides users of the EnviroTox database a transparent understanding of the variation between MOA classification schemes. In terms of regulatory relevance, an understanding of MOA, even at a simple binary level, can provide information that can infer chemical potency which can be useful for the prioritization (ranking) and risk assessment of chemicals. Here we present the results from the consensus MOA classification of 3900 organic chemicals according to the 3 classification categories described above: 40 and 17% of the chemicals are classified as Narcotics and Specifically acting chemicals respectively, whereas 43% cannot be classified. For 54% of the chemicals, the consensus MOA can be regarded as quite robust (medium to high confidence).

MO064

Application of Daphnia magna in toxicity assessment and detection of waterborne pathogens

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In this study, double staining method using two fluorescent dyes, Calcein AM and Propidium Iodide, was applied on *Daphnia magna* exposed to *Salmonella spp.* and *Shigella spp.* to assess their toxicity. Results showed that these bacteria caused disparate infectious ability on *daphnia* depending on age of this organism, bacteria concentration and exposure time. In detailed, *S. dublin* and *S. sonnei* are the most harmful strains to *daphnia*. Interestingly, older daphnia can give responses to nearly 10 CFU/ml of some bacteria strains. Besides, the proteomic profile of the treated *D. magna* was investigated using 2-dimensional gel electrophoresis method and then analyzed by the Progenesis software to explore the differentially expressed proteins (DEPs). The identified DEPs is involved in energy metabolism, oxidative stress response and dynamic structure of cytoskeleton filaments. The systematic approach to the analysis of proteomic responses used as well as the detailed analysis results has been useful in understanding the novel molecular mechanisms for pathogen induced pathological changes.Moreover, special DEPs can be used as novel sensitive biomarkers to detect these pathogens. Acknowledgment:\nThis work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No:PJ01267701)’ Rural Development Administration, Republic of Korea.

MO065
Automated, high-throughput measurement of size and growth curves of small organisms in well plates
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Organism size and growth curves are important biological characteristics. Current methods to measure organism size, and in particular growth curves, are often resource intensive because they involve many manual steps. Here we demonstrate a method for automated, high-throughput measurements of size and growth in individual aquatic invertebrates kept in microtiter well-plates. We use a spheroid counter (Cell^{3}iMager, cc-5000) to automatically measure size of seven different freshwater invertebrate species. Further, we generated calibration curves (linear regressions, all p< 0.0001, r^2>=0.9 for *Ceriodaphnoa dubia, Asellus aquaticus, Daphnia magna, Daphnia pulex;* r^2>=0.8 for *Hyalella azteca, Chironomus spec.* larvae and *Culex spec.* larvae) to convert size measured on the spheroid counter to traditional, microscope based, length measurements, which follow the longest orientation of the body. Finally, we demonstrate semi-automated measurement of growth curves of individual daphnids (*C. dubia* and *D. magna*) over time and find that the quality of individual growth curves varies, partly due to methodological reasons. Nevertheless, this novel method could be adopted to other species and represents a step change in experimental throughput for measuring organisms' shape, size and growth curves. It is also a significant qualitative improvement by enabling high-throughput assessment of inter-individual variation of growth.

MO066
Comparative toxicity of 20 nm Silver Nanoparticles (AgNP) and TiO2 Nanoparticles (TiO2NP) using CaCo2 cells
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Previous studies done by us indicated that AgNP at concentrations less or equal to 30 μg/ml were not cytotoxic nor inflammatory when exposed to human whole blood cultures (Kim L Lategan, Chavon R Walters, Edmund J Pool, 2019, The effects of silver nanoparticles on RAW 264.7 macrophages and human whole blood cell cultures, Frontiers In Bioscience Landmark Edition, 24, 347-365).The current study investigated whether CaCo2 human colon epithelial cells reacted similarly to AgNP, and to compare the relative toxicity of AgNP and TiO_2NP for these epithelial cells. CaCO2 cells were exposed to various concentrations of AgNP and TiO_2NP after which cell viability, and the secretory inflammatory biomarkers nitric oxide (NO), interleukin-6 (IL-6), interleukin-8 (IL-8) and macrophage migration inhibitory factor (MIF) levels were monitored. Cell viability was assessed using the XTT assay, NO using the Griess assay, and IL-6, IL-8 and MIF using commercially available DAS-ELISAs. Results obtained showed that AgNP is highly cytotoxic for CaCo2 cells (IC50 = 21 μg/ml). This study also showed that AgNP at 25 μg/ml had major modulatory effects on NO, IL-6, IL-8 and MIF secretion by CaCo2. On the other hand, TiO_2NP had only minor effects on cell viability, IL-6 and IL-8 at 250 μg/ml. Only NO and MIFsynthesis by CaCo2 were affected by concentrations of TiO_2NP below 250 μg/ml. In conclusion the current results show that AgNP has a major impact on CaCo2 cells at concentrations below 25 μg/ml. This is very different from our previously published data for AgNP using whole blood cultures. This indicate that there are differences in the adverse effects posed by the same size of AgNP for various cell types. On the other hand, TiO_2NP only had effects on CaCo2 cells at much higher concentrations. This indicate that TiO_2NP is less toxic than AgNP for CaCo2 cells. We suggest that future studies monitoring toxic effects of a specific nanoparticle should use a panel of cell lines or primary cells to get a wholistic view of adverse effects that may be caused by the nanoparticles.

MO067
Comparison of metabolism of fragrance chemicals in rainbow trout liver S9 sub-cellular fractions and in the invertebrate Hyalella azteca
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Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals which usually involves the determination of the bioconcentration factor (BCF) in fish. Two assays measuring *in vitro* intrinsic clearance rates of chemicals have been recently adopted as new OECD test guidelines (TGs) using hepatocytes or liver S9 sub-cellular fractions from rainbow trout to refine BCF predictions. Bioconcentration studies in the invertebrate *Hyalella azteca* had been recently proposed as an alternative non-vertebrate *in vivo* screening assay. One of the major uncertainties using an invertebrate as a surrogate for fish bioaccumulation may be encountered due to a different and limited metabolic capability compared to vertebrates. The goal of this study was to compare the metabolites formed for 3 different fragrance chemicals in rainbow trout assessed by the RT-S9 *in vitro* assay and in parallel *H. azteca*. BCF studies were performed with 3 different fragrance chemicals in a flow-through system with *H. azteca*. Concentrations of the parent chemicals in water and in the animal were determined by GC-MS analysis. Additional samples were prepared from exposed animals at two time points during the exposure phase for metabolite identification. Substrate depletion assays for the same chemicals were performed using RT-S9 following the new OECD TG319B. All samples were purified by SPE and metabolites determined using GC-MS and LC-MS-analysis. For all 3 fragrance chemicals hydroxylations as Phase I reactions followed by the formation of Phase II conjugates were observed both for RT-S9 and *H. azteca*. However, different kinds of Phase II conjugates were found for the two organisms. In RT-S9 conjugates with glucuronic acid were identified as major Phase II metabolites, whereas in *H. azteca* glucose conjugates were detected. Additionally, conjugates with sulfate or sulfate plus glucose were found in the invertebrate. Interestingly, a different preference in biotransformation for the different isomers of one of the fragrance chemicals was observed in *H. azteca* compared to RT-S9. Comparable metabolic routes, i.e. hydroxylation of hydrophobic chemicals followed by conjugation reactions using glucuronic acid or glucose and sulfate were observed for 3 fragrance chemicals in rainbow trout assessed in RT-S9 and in *H. azteca* indicating a high metabolic capacity which may render this invertebrate a suitable model for BCF studies as a novel screening approach.

MO068
Developing an Integrated Approach to Testing and Assessment for Acute Fish Toxicity Testing
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The assessment of aquatic toxicity is required in various regulatory frameworks and has a huge impact on the number of fish used in testing. The acute fish toxicity test (OECD Test Guideline [TG] 203), which uses lethality as the endpoint, is one of the most frequently used aquatic toxicity tests. This represents a very significant animal welfare concern. Moreover, while knowledge on the scientific limitations of individual fish tests in terms of reproducibility and relevance to environmental protection is growing, new scientifically advanced methods are enriching the toxicology toolbox. Thus, advanced Integrated Approaches to Testing and Assessment (IATA) for acute aquatic toxicity are of high social, environmental and scientific interest. IATAs may structure and guide combining mechanistic information from the molecular and cellular level such as from the RTgill-W1 cell line assay, information from *Daphnia* and algae as species of lower trophic levels and the Acute Fish Embryo Toxicity Test (FET, OECD TG 236) as refinement method. Through the OECD Test Guidelines Programme, Austria and the International Council on Animal Protection in OECD Programmes are working with leading experts and the OECD VMG-Eco, to develop such IATAs. The related OECD project aims to enrich the Threshold Approach for Acute Aquatic Toxicity Testing (OECD Guidance Document 126) with new toxicological tools, including computational approaches. Progress towards the development of IATAs will be discussed and one potential IATA will be outlined: Briefly, this IATA first considers all available data (e.g. physicochemical properties, mammalian toxicology, neurotoxicity and metabolism), data on the mechanism of action and from algae and *Daphnia* testing (OECD TGs 201 and 202). Facultative tiers may include *in silico* predictions for algae and *Daphnia* EC_{50} and for fish LC_{50}, experimental data from *in vitro* tests such as a cytotoxicity assays using fish gill cell lines, and the FET test. Mechanism of action information will inform the method selection within the IATA. No further testing is required once the fish EC/LC_{50} can be predicted or it can be concluded that algae or *Daphnia* are more sensitive than fish (embryos, cell lines). Testing according to TG 203 should only be performed as a last resort. *The views, conclusions and recommendations are those of the authors and do not necessarily represent the policies or positions of the organisations to which they are affiliated.*

MO069
Development of a rapid agar-based algae growth inhibition screening test
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The OECD guideline 201 "Alga, growth inhibition test" is routinely used in aquatic ecotoxicology to assess the toxicity of chemicals on phytoplanktonic species. The test is performed in liquid test medium incubated under controlled conditions for 72 hours. Range finding tests are usually performed to find the concentration range in which effects are likely to occur in order to subsequently

define the concentration of test substance inducing 50% reduction of growth (EC50). The present study aimed to apply rapid and cheap methods to assess algal growth inhibition that can be used as preliminary range finding test or as screening method during the chemical development phase. Algae or diatom cells are transferred from an exponentially growing liquid culture and inoculated in agar plates. Three methods are to be tested in order to choose the most appropriate one. A spotting assay consisting in transferring the test item to the inoculated plate with an inoculation loop. A paper disk approach where a 6mm filter disk with the desired test concentrations is applied in the inoculated agar plate. An agar well diffusion method using a cork borer to puncture a 6mm hole where 20-100 mL of test solution are going to be introduced. All plates were incubated facing up at 20°C to a continuous light exposure. Growth inhibition was monitored over 4 days and evaluated by the presence and/or absence of a radius of growth of inhibition (mm). The methods were used with the marine diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* the marine green microalgae *Tetraselmis chuii.* Results were compared with data obtained from the standard method (OECD 201). The method was used with the reference item 3,5-dichlorophenol and nanomaterials. The agar-based method is less labour intensive compared to the liquid method and can be adapted for freshwater algae species. We suggest it can be used as screening and/or range finding test in aquatic toxicity studies. **Acknowledgements**: This research is supported by the project SMARTAQUA that is funded through the MarTERA cofound partners Foundation for Science and Technology Portugal (FCT), Research Council of Norway (RCN) and Malta Ministry for Education and Employment (MCST)

MO070

Dioxin and AhR impairs cardiac development via direct regulation of key mesoderm genes in human embryonic stem cells

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Dioxin and dioxin-related polychlorinated biphenyls are potent toxicants with association with developmental heart defects and congenital heart diseases. However, the underlying mechanism of their developmental toxicity is not fully understood. Further, different animals show distinct susceptibility and phenotypes after exposure, suggesting possible species-specific effects. Using a human embryonic stem cell (ESC) cardiomyocyte differentiation model, we examined the impact, susceptible window, and dosage of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on human cardiac development. We showed that treatment of human ESCs with TCDD at the ESC stage inhibits cardiomyocyte differentiation, and the effect is largely mediated by the aryl hydrocarbon receptor (AHR). We further identified genes that are differentially expressed after TCDD treatment by RNA-sequencing, and genomic regions that are occupied by AHR by chromatin immunoprecipitation and high-throughput sequencing. Our results support the model that TCDD impairs human ESC cardiac differentiation by promoting AHR binding and repression of key mesoderm genes. More importantly, our study demonstrates the toxicity of dioxin in human embryonic development and uncovered a novel mechanism by which AHR regulates lineage commitment of ESC. It also illustrates the power of ESC-based models in the systematic study of developmental toxicology.

MO071

EcoToxChip: A toxicogenomics tool for chemical prioritization and environmental management

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Here we provide an overview of our Large-Scale Applied Research Program (LSARP) grant from Genome Canada that aims to develop, test, validate, and commercialize quantitative PCR arrays (EcoToxChips) and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. In Project Phase 1, EcoToxChips are being developed for laboratory model species representing the most important vertebrate groups in ecological risk assessment (fish-fathead minnow; bird-Japanese quail; amphibian-*Xenopus laevis*). Model species (adult and early-life stage, ELS) are being exposed via standardized tests to 8 environmental chemicals representative of natural resource/environment sectors of Canadian concern and also ones that impact a wide biological space (EE2, chlorpyrifos, benzo(a)pyrene, lead, fluoxetine, selenomethionine, trenbolone, HBCD) (Activity 1). An integrative systems approach based on functional 'omics (combined global transcriptomic and proteomic profiling, targeted metabolome) and physiological analyses across levels of biological organization is being applied to characterize relevant toxicity pathways including adverse outcome pathways, AOPs (Activity 2); from this, and other resources, species-specific EcoToxChips consisting of 384 environmentally-responsive genes of regulatory concern are being informed, built, tested, and optimized (Activity 3). EcoToxChip performance will be validated (and further optimized) through an inter-lab study with our collaborators (Activity 4). Under Activities 5-7, knowledge from Phase 1 is being translated to 3 native species (i.e., fish: rainbow trout; bird: double-crested cormorant; amphibian: Northern leopard frog). EcoToxXplorer.ca provides intuitive bioinformatics support and be modeled on our successful cloud-based tools (metaboanalyst.ca). To position the team advantageously with regard to the commercialization and institutionalization of the deliverables, our GE3LS research will produce and leverage social science knowledge about the phenomenon of "institutional entrepreneurship". The anticipated socioeconomic benefits associated with the adoption of our deliverables, namely more focused animal testing, improved regulatory decision-making, and cost-efficiencies. Here we provide a 30 month update of our project (www.ecotoxchip.ca).

MO072

Effects of PCB 28, PCB 52 and PCB 118 on Primary Mantle and Gill Cell Cultures of Unio pictorum and Unio crassus

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Polychlorinated biphenyls (PCBs) are among persistant organic pollutants under Stockholm Convention.They are widespread and hazardous contaminants of environment.Eventhough, their production has been banned since 1970s, they are still used in some industrial areas (paints, etc.), transfer fluids, capasitor oils and pesticides.European Food Safety Authority reported six PCB congeners (PCB 28, 52, 101, 138, 153 and 180) as indicator PCBs in the environment.Also, some PCBs are categorized as dioxin-like PCB (such as PCB 118) which is monitored in the environment.PCBs are easily transferred in the air, water, soil which can be then moved by dust, snow or rain in areas eventhough they are not used such as arctic. In the end of this cycle, they are a part of aquatic environment via bioaccumulating and biomagnificating in aquatic organisms or deposition in sediment.Thus, their persistance in aquatic environment make them potential treat to wildlife and eventually lead human health concerns.Due to the fact that they are filter-feeded organisms, mussel contain more concentrated PCBs in their tissues than water. Although there are a lot of studies, either *in vitro* or *in vivo*, that marine mussel species are used as indicator species, few studies are available for freshwater mussel species.Therefore, the present study was aimed to determine cytotoxic effects of two indicator PCB (PCB 28 and PCB 52) and one dioxin-like PCB (PCB 118) on primary mantle and gill cell cultures of two freshwater species *Unio pictorum* and *Unio crassus* using MTT assay. *U. pictorum* were found to be more sensitive compared to *U. crassus* for the mantle tissue for all the tested PCBs with lower IC50 values; this relation was not observed for gill cells.PCB118, the highest chlorinated congener among the tested compounds, showed least toxicity; which could be attributed to its molecular size where small molecules were able to solubilize and enter the cell easier to induce their toxic effects.The most cytotoxic compound was PCB28 on *U.pictorum* gill with an IC50 of 0.13 ppb; while the least was PCB118 in *U.crassus* gill with an IC50 of 47.37 ppb.Species specific differences along with the environmental conditions that the cultures *Unio* species are taken influence the *in vitro* toxicity effects.Meanwhile, this study provides valuable information on freshwater mussel species and their responses to environmental pollutants in *in vitro* conditions. Keywords: *Unio pictorum, Unio crassus,* PCB, cytotoxicity

MO073

Effects of Zinc Pyrithione and Copper Pyrithione on Primary Mussel Cell Cultures

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Antifouling paints are used against undesirable colonization of fouling organisms on natural and artifical surfaces; which impact economy by material loss and environment by spread of invasive species along with other undesirable health effects. Among the first used antifouling agent, tributyltin (TBT) was banned since 2008 due to its adverse effects on aquatic organisms and ecology. This resulted in the use of new alternative antifouling biocides such as zinc pyrithione (ZPT) and copper pyrithione (CPT). As these compounds in combination and alone, are still widely used, toxicity assays in the aquatic environment are needed for all possible affected species. As a freshwater mussel, *Unio crassus* Philipsson, 1788 (Bivalvia, Unionidae), is used as biological monitoring mollusc organism for freshwater ecosystems. Therefore, this study was aimed to determine the cytotoxic effects of ZPT, CPT (0,0008-3,125 µM) and their combination on primary gill and mantle cell cultures of *Unio crassus* using mitochondrial (MTT) and lysosomal (NR) cell viability assays.. According to results, ZPT were found to be more toxic than CPT. CPT alone application were found to be less cytotoxic compared to the combination where a decrease in IC_{50} of 90% and 81% (mantle and gill) were present for MTT assay. Both combination and alone applications indicate a cell death through mitochnondrial pathway compared to a lysosomal pathway. The results of study indicate that at nM concentrations, both zinc and copper pyrithrone have cytotoxic effects on the primary Unio mantle and gill cells; where the cytotoxicity increased by the combination of the tested drugs. Mantle and gill primary cultures provide useful biological monitoring tools to determine effects of environmental contaminants. Keywords: *Unio crassus*, cytotoxicity, zinc pyrithione, copper pyrithione

MO074
Endocrine disruptors screening analyzing zebrafish sperm quality
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Endocrine disruptors (EDCs) are chemicals that by interfering with the endocrine system can have an adverse effect at developmental, neurological, immune and reproductive level. The negative impact of EDCs is becoming a real public health issue, therefore the necessity of tests to assess the potential risk of new chemicals before they are marketed is increasing. The zebrafish is currently used as a model for the evaluation of acute and developmental toxicity, and also for the screening and testing of potential endocrine disrupters as described in the OECD Guidelines. However, the two major endpoints used to evaluate endocrine disrupting chemicals, vitellogenin concentration and change in sex ratio, have several limitations. With the purpose of expanding the number of tests available to identify xenobiotics with endocrine disrupting activity, we developed an assay that evaluates the reproductive performance of zebrafish (after *in vivo* or *in vitro* exposure to EDCs) by measuring sperm quality using a computer-assisted sperm analysis (CASA). For *in vivo* experiments, adult zebrafish (aged between 6-12 months) were exposed to heavy metals (copper or mercury), herbicide (N-[phosphonomethyl] glycine) and bisphenol A for a period ranging 2-15 days. At the end of the exposure period the testes were excised, placed in tubes with immobilization solution and the sperm was released by gently shake. For *in vitro* experiments, sperm from untreated adult zebrafish was incubated for 30 to 240 min with four concentrations of copper, methyl mercury, N-[phosphonomethyl] glycine and bisphenol A. In both experiments, sperm quality (after activation with system water) was evaluated using a microscope with a negative-phase contrast objective connected to a computer-assisted sperm analysis (CASA) system (Proiser R + D, S.L.; Paterna, Spain). Parameters such as progressive motility, curvilinear velocity and linearity were evaluated and compared to DMSO treated control. The assay here proposed with these two *in vitro* and *in vivo* approaches, can discriminate between compounds with a direct cytotoxic effect (reducing sperm motility) and compounds that might generate, after long exposure, a reduction in fertility by disrupting the overall process of spermatogenesis.

MO075
Enhancing the utility of the Sequence Alignment to Predict Across Species Susceptibility tool
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The number of new chemicals introduced to commerce each year exceeds the practical ability to experimentally determine the consequences of those chemicals on all species that may be exposed. To address this challenge new approach methods are increasingly becoming important as supplementary evidence for chemical safety evaluations. A new approach method for predicting cross-species chemical susceptibility is the Sequence Alignment to Predict Across Species Susceptibility tool (SeqAPASS v3; https://seqapass.epa.gov/seqapass/). SeqAPASS is a web-based tool that allows the user to begin to understand how broadly toxicity information may plausibly be extrapolated across species, while describing the relative intrinsic susceptibility of different taxa to chemicals with known modes of action (e.g., pesticides, pharmaceuticals, other specifically acting chemicals). The tool rapidly and strategically assesses available molecular target information to describe protein sequence and structural similarity at the primary amino acid sequence, conserved domain(s), and individual amino acid residue levels. Development of the SeqAPASS tool is an iterative process making improvements by gathering information from users through training and outreach to identify new functionality and user-friendly features for implementation. Such features are anticipated to grow the user base and improve susceptible predictions. Recently the SeqAPASS development team has begun to integrate help menus and tools tips, as well as features for interoperability with other tools (e.g., ECOTOX Knowledgebase; https://cfpub.epa.gov/ecotox/) for the next release of SeqAPASS v4 anticipated for 2019. Additionally, it is critical to continue to continue to demonstrate the utility of the SeqAPASS tool in predicting cross-species chemical susceptibility. Therefore, a case study was developed considering cross-species susceptibility to diamide insecticides.

MO076
Evaluation of skin mucus vitellogenin (VTG) in a Japanese Medaka fish sexual development test (OECD TG 234) with 17beta-ethinylestradiol (EE2) as contribution to 3Rs animal welfare concept
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Background: Vitellogenin is regarded as sensitive biomarker to detect exposure to endocrine active chemicals in fish and provides mechanistic mode-of-action information. The current OECD TGs 229, 234 and 240, recommend measuring VTG in the liver or head/tail homogenate of sacrificed fish. In the OECD TG 240 an extra cohort is used for whole body VTG measurement. VTG measurement in skin mucus of living fish is possible and may save individuals for other investigations. **Objective:** Implementing VTG measurement in skin mucous in an OECD TG 234 positive control study and comparison to established recommended procedures. **Methods:** The fish sexual development test was performed generally according to the OECD TG 234 with two replicates of 30 fertilized eggs each, in the control group and in the treatment group (0.1 μg/L EE2) in a flow-through system. Medaka fish (*Oryzias latipes*) were raised until day 60 post hatch. All recommended endpoints were measured. This poster focuses on secondary sex characteristics (stereomicroscopic investigation after modified Davidson's fixation of the dorsal and anal fin), genotypic sex ratio (dmy gene with PCR), VTG levels in skin mucus and head/tail homogenate (ELISA) and histological evaluation of gonads, liver, and kidney in the midsection cut after fixation in Davidson's solution. The VTG measurement in skin mucous, whole fish body and liver tissue had been validated in advance. **Results:** Whereas in the control group the phenotypic sex ratio was 58/42 % (male/female), in the EE2 group all fish were phenotypically females as confirmed by histologic investigation of the gonads. The genotypic sex ratio in the control group matched the phenotype and the genetic sex ratio in the EE2 group was not statistically different to the control. Female fish from the control and EE2 group could be differentiated from male controls by VTG levels in skin mucus as well as in head/tail homogenate. However, the coefficient of variation in each group was higher among the VTG in mucus compared to the head/tail VTG. **Conclusion:** Vitellogenin in skin mucus can be used as parameter especially for in interim measurements from living fish during the course of a study. However, the mucus sampling procedure should be further standardized to minimize variation and increase the power of this parameter.

MO077
Extending International Guidance to Relevant Middle Eastern Organisms: Acute Exposure of 4 Aromatic Hydrocarbons to Indigenous Marine Species Using a Passive Dosing Approach
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The Arabian Gulf is a dynamic environment with unique habitats. The inhabitants of this marine ecosystem have been understudied and a lack of reliable data exists to benchmark their sensitivities against commonly used test organisms. Current testing guidelines do not reference relevant species for this region of the world. Recent efforts are underway to develop guideline extensions for relevant species from primary trophic levels. An Indigenous copepod, algae and juvenile killifish were selected as model test organisms to perform acute exposures of 1-Methylnaphthalene, Phenanthrene, Biphenyl and Octahydrophenanthrene. Test durations were 48, 72 and 96 hours and generally followed OECD 158, 201 and 203 respectively. To deliver and maintain controlled exposure concentrations, a passive dosing format consisting of silicone O-rings was employed. Critical Target Lipid Body Burdon's for similar species were used as inputs in The Target Lipid Model to select a range of 5 concentrations and subsequently benchmark sensitivity differences against other species currently recommended within international guidelines (i.e. OECD, ISO, EPA, etc.).

MO078
In vitro and in silico evaluation of antiproliferative activity and receptor binding affinity of newly synthesized succinimide derivatives
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The succinimide moiety provides a pharmacophore for potential antiproliferative agents. Their potential affinity to bind to estrogen receptors may be important for their antiproliferative activity against estrogen positive cells as well as for their endocrine disrupting potential. The aim of this study was to determine in silico estrogen receptor binding affinity, in vitro antiproliferative effect against estrogen positive and estrogen depending cells of six newly synthesized succinimide derivatives and to find the possible in vitro-in silico association of their affinity toward estrogen receptors. Six newly synthesized compounds were evaluated for their in vitro antiproliferative activity toward cervix carcinoma (HeLa) and estrogen positive human breast carcinoma (MCF-7) cell lines. Standard MTT assay was applied to evaluate cytotoxic activities of the analyzed compounds after cells were exposed for 48 h. For each compound IC_{50} was calculated. Moreover, for each compound *Estrogen Receptor alpha* (logRBA) affinity (www.acdlabs.com) were calculated. Five compounds expressed antiproliferative power against HeLa. Introducing fluorine in the molecule in para position was followed by 20-fold increment of antiproliferative effect and decrement of IC_{50}.

However,substitution of fluorine with chlorine and bromine induced increment of IC_{50} against HeLa cells. Position change of a chlorine and bromine from para to meta nulled the cytotoxic effect of the molecules. In silico for all observed compounds was detected modest *Estrogen Receptor alpha* (logRBA) affinity which varied from 0.12 to 0.35. However, statistically significant parabolic association was obtained between in silico determined logRBA and IC_{50} against HeLa cells (r^2=0.98, p< 0.01). Only three compounds have demonstrated antiproliferative capacity against MCF-7 cell lines. Hence, there were not enough in vitro data about affinity towards estrogen positive carcinoma cells to support the in silico obtained results. Strong parabolic in vitro-in silico association of antiproliferative activity of newly synthesized succinimide derivatives against HeLa cells and their predicted estrogen binding affinity was determined. *Acknowledgment:* This work was performed within the framework of the research projects No. OI 172013, supported by the Ministry of Education, Science and Technological Development of Republic of Serbia.

MO079

In vitro test to detect the presence of neurotoxic compounds in water and sediment samples

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In this work, an *in vitro* test for the detection of compounds with neurotoxic effects in water and sediment samples is proposed, since *in vitro* tests have been considered as an alternative to animal tests, in order to comply with the proposal of the 3 Rs (Replace, Refine and Reduce). In addition these techniques have the advantages of having a better control in the conditions in an experiment, reduce the ethical implications, and are of lower cost. In this study a method was developed using mollusc foot tissue (*Pteria sterna*), exposed to pollutants to evaluate the activity of the AchE enzyme, to detect the presence of compounds with neurotoxic effects. Tests were carried out with metals (Cd and Cu), surfactants (LAS) and pesticides (Dichlorvos and Cypermethrin), to evaluate the response of this test to these contaminants. The tissue homogenates were prepared with phosphate buffer (pH 7.2), then the homogenate was incubated with the xenobiotics for 30 min. Later the activity of the AchE enzyme was determined by the Ellman method (microplate). The results obtained with the pollutants were significantly different from the tests without pollutants. In the tests with cadmium (0.05, 0.1, 0.5 and 1.0 mg L^{-1}) the inhibition of Ache enzyme activity varied from 8.5%, to 22.4%. In the tests with detergents (0.05, 0.1, 0.5, 1 mg L^{-1}) from 11.5 to 32.7% and in the tests with pesticides (0.001, 0.005, 0.05, 0.5 mg L^{-1} from 23.8 to 86.5% in all cases the response of this *in vitro* test was according to the concentration of the xenobiotics tested.

MO080

Lipophilicity influence on estrogen binding affinity of newly synthesized succinimide derivatives

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Design of new drug entities beside biological activity and defined pharmacokinetic properties, also should have low environmental especially low potential to disturb the normal endocrine system function. Lipophilicity is the key phisicochemical property which defines the half-life time in the environment and toxicity for biotic and abiotic environment. The aim of this study was to determine in silico estrogen receptor binding affinity as well as their lipophilicity by use of chromatographic methods for six newly synthesized succinimide derivatives and to observe the lipophilicity influence on their affinity toward estrogen receptors. RP TLC was used for study of the retention behavior of N-(3- or 4- substituted phenyl)-2-phenyl-succinimide derivatives. Precoated RP-18W/UV254 10×10 cm plates (Macherey-Nagel GmbH and Co., Düren, Germany) were used as stationary phase and binary water-acetone solutions with a varying volume fraction, φ (0.45-0.70) of organic solvent, were applied as the mobile phase. After development, the spots were detected at 254 nm with UV lamp and R_f values were measured. For each analyzed compound *Estrogen Receptor alpha* (logRBA) affinity (www.acdlabs.com) and ClogP were predicted. Retardation factor, R_M was calculated for each compound according to the equation: $R_M=\log(1/R_f-1)$. Furthermore, in the linear correlation of the R_M values versus φ, the chromatographic retention constant R_M^0, was obtained as extrapolated value to 0% point: $R_M=R_M^0+S\times\varphi$. Highly significant linear correlation between in silico determined ClogP and experimentally obtained retention constants R_M^0 (r^2=0.972; p< 0.001) as well as against slope, S (r^2=0.954; p< 0.001) was observed, respectively. Hence chromatographic parameters may be used as lipophilicity measure. Modest in silico *Estrogen Receptor alpha* (logRBA) affinity which varied from 0.12 to 0.35 was calculated for the analyzed compounds. Statistically significant linear correlation was obtained between logRBA and lipophilicity determined as R_M^0 (r^2=0.857; p< 0.01) and S (r^2=0.904; p< 0.01), respectively. Lipophilicity increment of newly synthesized succinimide derivatives may enhance affinity for estrogen receptor alpha and thus increase their potential to affect endocrine system functions. *Acknowledgment:* This work was performed within the framework of the research projects No. OI 172013, supported by the Ministry of Education, Science and Technological Development of Republic of Serbia.

MO081

Measuring oxidative stress in zebrafish cell lines via a novel in vitro assay

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Establishing *in vitro* methods is considered a high priority, since most risk assessment relies on in *vivo* studies. The Swedish Research council - amongst other European institutions - is increasingly funding programs dedicated to 3R principles. Therefore, we started our project “toxicity pathways – a novel strategy to reduce and replace *in vivo* studies in fish”. It is the prescribed goal to establish a battery of stably transfected *D.rerio* cell lines in order to test for specific toxicity endpoints. The latter may then be used for omics-based high throughput applications, screening, and prioritizing of positives. First, transfection efficiency of commercially available transfection reagents (Mirus X2, LT1, 2020; Promega FHD, F6, Viafectamine, TransFast; Roche XHP; Invitrogen Lipofectamine 2000; Qiagen SuperFect, Effectene) were tested in Pac2, ZF4, and ZFL *D.rerio* cell lines. Most promising candidates (FHD for Pac2 and ZF4, XHP for ZFL) were selected for cell transfection in transient reporter gene assays. A Nrf2-sensitive pGL4-based firefly-luciferase expressing plasmid was co-transfected with a normalizing renilla-luciferase plasmid. Second, all three reporter lines were exposed for 24h to exponentially increasing concentrations (0.1, 1, 10, and 100 μM) of known oxidative stress inducing chemicals (tertButylquinone, peroxide, and sulforaphane). In parallel, cell-viability was scored using standardized MTS-assay. Dose-response relationships could be shown in all transiently transfected cell lines for tertButylquinone and sulforaphane. Concentrations of causing less than a 20% decrease in cell-viability were considered as non-cytotoxic and should be used in ongoing assays for receptor activation or inhibition. ZF4 and ZFL were prioritized for further use. Last, a panel of environmentally applied pesticides (diazinon, deltamethrin, atrazine, metazachlor, tertButhylazine, diuron) was used for 24 h exposure (6.25, 12.5, 25, 50, 100 μM) of Nrf2 transiently transfected cells, in order to test for the ecotoxicological applicability of the bioassay. Dose response relationships were shown for certain tested pesticides (metazachlor, deltamethrin, diazinon), proofing the robustness of the assay.

MO082

Prediction of Acute Ecotoxicity of Chemical Substances by Graph Theory Approach

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Prediction of ecotoxicity of chemical substances catches much more social concern again in recent years. Most of the conventional methods such as ECOSAR is clustering the chemical substances empirically from the structural information of chemical substances and then predicting the toxicity value by employing a logP involved linear regression model (QSAR formula). Besides this, most of the conventional methods are not appropriate for predicting the ecotoxicity of inorganic compounds. Due to the nature of empirical clustering, the prediction accuracy would not be improved even if new toxicity test data are added. In addition, the user will get puzzled in selecting the prediction result in the case of multiple QSAR formulas with a chemical substance. To overcome the flaws of the existing methods, we employed the big dataset of AIST-MeRAM ecotoxicity test data and the structural information of Maccs Keys that vectorize a chemical structure to the 166-bit binary information, applied the graph theory to the dataset and to investigate the self-clustering by various unsupervised machine learning methods. We finally developed a new method in which the acute toxicity of fish, daphnids, and algae can be predicted with good accuracy, while the LogP or linear regression model in existing methods were not further necessary. To confirm the prediction ability of the new method, cross-validation and comparison with the prediction results of ECOSAR was performed. The prediction ability is proved to be equivalent or better than that of the existing methods, and a wider variety of chemical substances could be coped. In addition, this new method is flexible to accepting toxicity test data and thereby the prediction accuracy could be improved.

MO083

Quality criteria for the development and application of (Q)SARs and further in silico methods in order to increase regulatory acceptance among various regulatory frameworks

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In order to meet data requirements for human and environmental safety assessment required by several chemical regulations, *in silico* methods pose an efficient alternative to traditional experimental testing, as they enable faster generation of data, help to avoid animal testing, and minimize costs as well as other resources. *In silico* methods describe computer-based calculation models

based on the hypothesis that similar structural features lead to similar effects or properties. They comprise several non-testing methodologies, such as (Q)SAR models, grouping approaches and expert systems. Just like experimental methods, *in silico* approaches have limitations in terms of areas of applicability. Indeed, (Q)SAR models are typically restricted in their applicability depending on the chemical frame, in which they have been developed, i.e. there is no "one model fits all" approach possible. In consequence, the evaluation whether a target molecule fits within the applicability domain of a (Q)SAR model, is a key aspect for assessing the reliability of a prediction. Although there is certainly, an increasing interest to use *in silico* methods on industry and regulatory bodies, acceptance of *in silico* predictions for regulatory hazard assessment is not guaranteed and users may rather prefer experimental testing as a safer choice. In order to improve standardization and harmonization of *in silico* methods and decrease the uncertainty for both regulatory bodies and industry, several guidance documents have been released in the last years in various regulatory frameworks [1, 2]. On the other hand, to date no criteria or guideline for the regulatory acceptance of (Q)SARs has been developed and guidance and instructions have not been adopted to a sufficient extent in every sector. This poster will address in more detail the current situation on quality criteria and acceptance of *in silico* methods for hazard assessment in different regulatory frameworks. It will furthermore describe what the different actors ((Q)SAR developers, authorities, regulatory bodies and industry) have to consider and improve in order to overcome the barriers of acceptance of computational assessment methods.
References: [1] ECHA. 2016. Practical Guide: How to use and report (Q)SARs. European Chemical Agency. [2] NAFTA TWG. 2012. (Q)uantitative Structure Activity Relationship [(Q)SAR] Guidance Document. North American Free Trade Agreement (NAFTA), Technical Working Group on Pesticides (TWG), 186.

MO084
Skin sensitization assessment for agrochemicals - a suggested approach assessing the applicability of non-animal test methods/approaches and global acceptance
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Non-animaltest methods/approaches for assessment of skin sensitization potential are available for chemical substances but can these be applied to agrochemicals and can a common global strategy be employed for their registration? For the recent REACH 2018 registration deadline for industrial chemicals, a non-animal test methods/approach was utilised. The availability of existing data for a weight-of-evidence assessment to determine testing strategy was firstly considered. *In silico* assessments of substances using (Q)SAR models to see if probability and/or alerts for skin sensitization are raised were also employed. *In chemico/in vitro* tests are required as an alternative to animal testing, addressing (where possible) key events 1, 2 & 3 of the skin sensitization Adverse Outcome Pathway (AOP). Currently, three non-animal OECD test guidelines are validated and regulatory-accepted for skin sensitisation assessment: *In Chemico* OECD 442C DPRA (key event 1) *In Vitro* OECD 442D either the ARE-Nrf2 Luciferase Assay (KeratinoSens™) or the ARE-Nrf2 luciferase LuSens test method (key event 2) *In Vitro* OECD 442E either the human cell line activation test (h-CLAT) or the U937 cell line activation Test (U-SENS™) or the Interleukin-8 Reporter Gene Assay (IL-8 Luc assay) (key event 3) *In vivo* testing (e.g. LLNA) may be performed from the start for some substances, but only if certain criteria apply. Moreover, testing *in chemico, in vitro or in vivo* may not need to be conducted in some circumstances. How do you know which criteria apply to your substances? In the flowchart we present a tiered approach to meet CLP classification requirements for your substance. We also review the results from applying the testing strategy to industrial chemicals for the REACH 2018 deadline and look at the concordance on acceptance of *in vitro* testing alternatives/approaches globally for agrochemicals.

MO085
Strategically Selecting Test Species using the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) Tool
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Chemicals in the environment can affect the health of wildlife with the potential for vastly different sensitivities among species. However, toxicity data used in risk assessment is based on a small number of model test species which might not represent the diversity of species sensitivities. This uncertainty is particularly true for many contaminants of emerging concern (CECs), including agonists of the peroxisome proliferator-activated receptor y (PPARy), for which species-sensitivity information might only be available for one or a couple species. However, performing toxicity testing for many species to determine the range in sensitivity is not practical. Therefore, the goal of this study was to demonstrate a means of selecting test species in a strategic fashion to determine the extent of differences in sensitivity by using the least number of species possible. Specifically, this study used the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool to rapidly and computationally predict species-specific chemical susceptibility across phylogenetically diverse species through evaluation of structural similarities and differences in the protein target of a chemical. The public literature was used to identify 17 key amino acid residues in the ligand binding domain of PPARy that interact with chemicals, along with knowledge of amino acid differences that cause differences in binding affinity. Differences in these amino acids were investigated among sequences of 246 phylogenetically diverse species from the NCBI database using SeqAPASS. Four amino acid positions had differences across species that were predicted by SeqAPASS to potentially result in differences in binding of chemicals and therefore potentially result in differences in sensitivity among species. Five PPARy-types were proposed that likely differ in their sensitivity to agonists, namely Type 1 (mammals), Type 2 (birds, reptiles, amphibians, ancient fish), Type 3 (most fish), Type 4 (salmonids), and Type 5 (zebrafish). Based on these results, Xenopus (Type 2), fathead minnow (Type 3), rainbow trout (Type 4), and zebrafish (Type 5) were strategically selected as being representative of the diversity of species sensitivities to agonists of PPARy for ongoing investigation. This study demonstrates how SeqAPASS can be used to computationally predict species most likely to differ in sensitivity to chemicals for the strategic characterization of species differences in sensitivity.

MO086
Toxicogenomic analysis of Akaki river water using Caenorhabditis elegans
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Deterioration of water quality is a threat to human and animal health worldwide. The problem is more overwhelming in developing countries where river water in urban regions serves as a final effluent point for industries and municipal discharge. Akaki river is the major river running through the center of Addis Ababa, Ethiopia and the downstream residents use the polluted river water for domestic and agricultural purposes. Previous studies of the Akaki river simply measured the levels of a limited number of individual pollutants. Toxicogenomic analysis using the *Caenorhabditis elegans* nematode offers an attractive model for measuring biological effect of the diverse pollutants.Water samples were collected from two sites in the Akaki river; MK (residential area) and BA (industrial area). Synchronized L1 *C. elegans* were exposed to the water samples or K-medium (control) until L4/young adult. The gene expression responses were evaluated by qPCR analysis of 30 genes associated with heat shock response, oxidative stress, innate immunity, metal response and development. Akaki river water induced a significant change in gene expression in exposed *C. elegans.* Although the levels of almost all analyzed metals and organic pollutants were below the permissible limit, the compiled effect resulted in transcriptional changes at the molecular level. Exposure to the waters led to an increased expression of *hsp-16.2*, *hsp-16.48*, *hsp-16.1*, *vit-6*, *daf-12*, *sod-1*, *tir-1*, *clec-60*, *tol-1 cyp-35A2* and *numr-1,* whereas the expression of sip-*1*, *lys-7, pgp-5*, *daf-16*, *lys-7, abf-2* and gst-*4* were significantly decreased compared to the control. A significant spatial variation was observed between the two sites in the expression of certain genes. The study demonstrated the usefulness of nematode based toxicogenomics analysis as an alternative tool to identify complex mixtures of pollutants and that Akaki river elicits a toxic effect on *C. elegans* at the molecular level.

MO087
SETAC Animal Alternatives in Environmental Science Interest Group
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Target and NonTarget Mass Spectrometry for Human and Environmental Exposure Assessment (P)

MO088
Identification of chemicals of emerging concern using HRMS data: framework for suspect screening and effect-directed analysis
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A generic framework is proposed to identify and prioritize emerging chemicals based on their exposure levels and toxicological profile for future regulatory and

screening efforts. This framework incorporates both suspects screening in untargeted high-resolution mass spectrometry (HRMS) data and effect-directed analysis (EDA). Untargeted mass spectrometry screening facilitates the measurement of thousands of small molecules in the environment and in human biological samples. Subsequently, a fast but putative identification can be acquired by comparing the accurate mass of a measured small molecules with the accurate mass of a known compound, an approach that is also embedded in effect-directed analysis. The first stage of the framework incorporates a database that contains accurate masses of >70.000 chemicals and their metabolites, this database can be used to putatively identify emerging chemicals in untargeted HRMS data, through a suspect screening approach. Further validation of putative matches can be done using newly developed bio-informatics tools, assessing MS/MS spectra, or using standards. Subsequently, in stage two of the framework their toxicological profile can be predicted based on their structure using computational tools (e.g. QSARs). EDA is a combination of biotesting, fractionation and chemical analysis and is used as a bottom-up approach where first the biological activity is characterized for different fractions of the sample. Subsequently, chemical analysis is performed on the bioactive fractions to identify the chemicals responsible for this activity. The method that will be used to measure bioactivity is the transthyretin (TTR) binding assay (TTR-FITC-T4 assay). This assay measures the interference of a chemical by competition with T4 in the binding to TTR which is important transport protein in the thyroid hormone system. Using a downscaled assay format in combination with increased fractionation resolution achieved by ultra-performance liquid chromatography (UPLC), high-throughput analysis of large numbers of samples is made possible. EDA will be done complementary to the suspect screening to identify those emerging chemicals that have a specific toxicological effect which were not primarily considered with suspect screening. The combined information about probability of presence in human samples and probability of toxicity will be used to rank emerging chemicals for prioritization in future human biomonitoring programs.

MO089

Identification of parent and TP compounds in waste water effluents by non-target analysis

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The search of CECs in aquatic compartment has always been a run-up to the new emerging substance or "never studied before" class: from PPCPs to PFAS, nanomaterials and so on. Despite the immense number of chemicals in production and in use, our attention is still restricted to only a small number of well-known contaminants. However, are we sure that this target approach is correct to study chemical pollution and to protect environment? Isn't like focusing on the top of an iceberg and ignoring the hidden part? With innovative LC-HRMS instruments, and *full scan* analysis, one water sample measurement can return many thousands of signals that correspond to further molecules. One analytical run becomes a frame of the sample, because peak detection is not limited, but at the same time, it generates about 1 GB or more of data. Develop a model to prioritize peaks, simplify data elaboration and molecule name assignment, is the aim of the study. This non-target method was applied to wastewater samples, in particular to micropollutants characterised by persistence and formation during treatment plant processes, likely environmentally relevant.\n24-h composite samples were collected from a WWTP influent and effluent for 7 consecutive days. Water samples were filtered by glass fiber filter (0.7 μm) and extracted on OASIS HLB (Waters) cartridge. The initial LC-HRMS analyses were performed in full scan conditions (100-1000 m/z) with positive and negative electrospray ionization in separate runs. Different chromatographic gradient conditions were selected: methanol/ammonium formiate buffer for positive ESI mode and methanol/water and methanol/ammonium formiate buffer for negative ESI mode. Workflows for the prioritization and identification of the non-target peaks were carried out with Compound Discoverer 3.0 (ThermoFisher). A first prioritization was done after correction of the responses for matrix suppression by internal standards: RT alignment, blank subtraction, minimum peak intensity. Secondarily, the filtered compounds were analysed with the same chromatographic set up but with a data-dependent approach, to collect MS/MS information. Potential parent compounds and TPs in effluent samples were identified both using by suspect screening than by identity and similarity approaches.

MO090

Identification of organic pollutants and their transformation products in Upo wetland via target, suspect and nontarget screening using LC-HRMS

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Diverse organic pollutants have occurred in natural water systems (e.g., wetland) and disrupted the aquatic ecosystem. Wetland would play a role as a melting pot of environmental pollutants promoting their degradation and elimination. Upo, the largest inland wetland in Korea, is mainly surrounded by agricultural area and so contaminated by diverse organic pollutants, especially pesticides. To extend our knowledge on contamination and decomposition functions of the wetland, major organic pollutants and their transformation products in Upo were traced by target, suspect, and non-target screening method using LC-HRMS. The wetland samples were collected at 8 different sites in November, 2017. As a result of first suspect and non-target screening to identify major pollutants, total 11 compounds were tentatively identified, including 8 fungicides (azoxystrobin, boscalid, carbendazim, cyprodinil, metalaxyl, methabenzthiazuron, tebuconazole, tricyclazole), 2 herbicides (atrazine, hexazinone) and 1 insecticide (thiacloprid). Among them, azoxystrobin, hexazinone, metalaxyl and tebuconazole were major pollutants in the wetland and confirmed by target analysis with reference standards. The median concentration of major pollutants was 46 ng/L for azoxystrobin, 55 ng/L for hexazinone, 220 ng/L for metalaxyl and 1,200 ng/L for tebuconazole. Some transformation products or metabolites such as azoxystrobin acid, metalaxyl acid, and 2-aminobenzimidazole were also tentatively identified. The peak intensity ratio of TPs to their parents gradually increased along with stream current direction of wetlands and greater up to 3 times at a downstream site. This suggests that the wetland water system serve as a degradation spot for organic pollutants. In future studies, quantitative analysis is needed for tentatively identified compounds in order to quantitatively evaluate the transformation kinetics and degradation capability of the wetland

MO091

Smart target method development for detection of antiviral compounds in aqueous environmental samples based on suspect screening and HRMS

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Pharmaceuticals and other chemicals used worldwide are present in the environment because of the human impact. After a worldwide outbreak of swine influenza virus in 2009 antiviral drugs have been researched more intensively as environmental pollutants. But, antiviral drugs used for treatment of other viral infections like HIV, hepatitis or herpes could be equally significant since there are 2.1 million new HIV infections being reported (for 2015, source UNAIDS). This number has been in moderate decline over the last decades, however still today, over 36 million people worldwide are living with HIV. In the human body, they are only partially metabolised and, once excreted, they can reach the aquatic environment via WWTPs. As a result, they can end up in surface water together with the mixture of their human metabolites and transformation products which can form along the way. The aim of this work was to evaluate systematically their presence in the aquatic system. In order to avoid classical target method development which commonly relies on arbitrary selection of target compounds, here we used a suspect screening approach where a 100+ antivirals suspect list was matched to WWTP samples as a guarantee of environmental presence. This was especially advantageous since no data on drug consumption could be obtained. In our case, WWTP influent and effluent of the largest catalan WWTPs was sampled over three consecutive days. The samples were extracted using a generic SPE method and analysed using LC-QExactive-MS. Each positively detected exact mass was further rationalised with fragmentation patter study for a specific compound/structure. A list of antivirals identified and detected in all three consecutive days were considered important and were advanced to the target method development step. The list was joined with compounds that have been reported to occur in high concentrations in other publications in order to check whether non-detection was due to lack of presence of lack of selectivity. Additionally, recently marketed compounds were also added to the method since its consumption rates were expected to grow over time. Finally, target method was developed using SPE-high resolution LC-MS for fast and reliable quantification of forty antiviral compounds in the aquatic environmental samples.

MO092

Suspect and non-targeted strategies to investigate in vitro and in vivo biotransformation products of emerging environmental contaminants: the benzotriazoles and benzothiazoles

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Benzotriazoles (BTRs) and benzothiazoles (BTHs) derivatives are high production volume chemicals involved in a wide range of applications and consumer products. The widespread use of BTRs and BTHs and their occurrence in a wide range of environmental media (drinking water, indoor and outdoor air, indoor dust, textiles, etc.) as well as human urine samples across different countries suggest that the population is exposed continuously to these chemicals worldwide. However, while human exposure has been observed, exposure assessment to these chemicals is very limited and it is only based on the analysis of parent compounds in human specimens. The objective of this study was to investigate the *in vitro* human Phase I and Phase II biotransformation of six emerging contaminants (3 BTRs and 3 BTHs) through cytochrome P450 (CYP), uridine glucuronic acid transferase (UGT), and sulfotransferase (SULT). Highly used and ubiquitous BTRs and BTHs were selected as substrates in this present study. Moreover, they were considered as model compounds to predict potential biotransformation products of other BTRs and BTHs derivatives. Generated biotransformation products in the samples were investigated using liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS), followed by their identification and structural elucidation based on a workflow combining suspect and non-target strategies. Most of the proposed biotransformation products were identified and structurally elucidated for the first time and possible metabolic transformation pathways were presented for the studied compounds. The identified biotransformation products were then targeted for analysis in human urine samples. Our findings provide important insights for the selection of biomarkers in future biomonitoring studies and emphasize the importance to investigate the biotransformation products in order to assess overall exposure to xenobiotics.

MO093

Identification of chemical compounds enriched in the Baltic sea surface microlayer using targeted and non-targeted liquid chromatography mass spectrometry

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The global oceans are known to be recipients of many pollutants but they can also act as a source of pollutants back to the atmosphere via sea spray aerosol (SSA). Although previous research has demonstrated that SSA is enriched in organic matter, a mechanistic understanding of what types of chemicals enrich in SSA is currently lacking. Results of our previous lab experiments suggest that enrichment in the sea surface microlayer (SML) is a good proxy for enrichment in SSA, although SSA enrichment factors (EFs) can be orders of magnitude larger than the corresponding SML EFs. Here, we use field samples to explore what types of biogenic and anthropogenic substances enrich in the SML. Eight paired bulk water and SML samples were collected in the Baltic Sea in June of 2018. Unfiltered samples were concentrated by a factor of 1000, using solid phase extraction. Analysis was performed by liquid chromatography high-resolution mass spectrometry on a Quadrupole-Orbitrap mass spectrometer. A targeted method was applied to determine SML EFs for a set of known environmental pollutants of low and high surface activity: pharmaceuticals and perfluoroalkyl acids. Additionally, a non-targeted screening method was applied to identify “unknown” chemicals which enrich in the SML. All samples were first analysed in fullscan mode. Peak areas were used to calculate SML EFs for each exact mass in each sample pair. A condition for the successful use of this data reduction approach is that any matrix effects are similar in both sample types (bulk water and SML). Analysis of spiked samples demonstrated that this was true for >95% of the test compounds. The screening detected 960 substances which had SML EFs >1.2. Of these, 680 substances were detected in positive ionisation mode and 280 in negative ionisation mode. After reanalysis in MS/MS mode, tentative identification via a mass spectral database was possible for 9 of the substances (>90% similarity). Further processing to achieve identification is currently performed on the 229 substances which had SML EFs >2. This involves scrutiny of fragmentation patterns, as well as using tools to identify homologue series. The structures of tentatively identified substances will be confirmed by comparison with chemical standards.

MO094

Development of an Ultrahigh Resolution Mass Spectrometry-Based Method for Nontarget and Suspect-Screening of Nitroaromatic Compounds in Atmospheric Particulate Matter (PM2.5)

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Air pollution is an environmental challenge in modern societies and a major cause of disease and premature death worldwide, with disproportionate consequences in developing countries. Ambient fine particulate matter ($PM_{2.5}$) and associated nitroaromatic compounds originate from combustion processes. Nitroaromatics are present in most atmospheric samples, are highly toxic and have a significant impact on climate forcing due to their light absorbing properties. Existing methods for monitoring of nitroaromatic compounds in the ambient atmosphere rely on targeted methods for specific compounds, but $PM_{2.5}$ is a complex mixture that is largely uncharacterized, thus here we developed and applied a method based on HPLC-ultrahigh resolution mass spectrometry. It was hypothesized that the highly diagnostic fragmentation and neutral loss behavior of nitroaromatics could be used to develop a comprehensive screening method for known and unknown compounds. Model compounds, including mono-nitroaromatics and poly-nitroaromatics (i.e. nitro-PAHs) were first analyzed to optimize analytical conditions. The optimal method was then applied to a series of $PM_{2.5}$ collected on quartz fiber filters during a campaign (SAPOEX18) in the Maldives between January and April 2018. Back trajectories were calculated for each sampling period using the Hysplit model, and 15 sample extracts were analyzed from three group defined by their geographical source regions: Bangladesh, India, and Arabian Sea. The nitroaromatic profiles will be discussed with respect to frequency of detection, meterological conditions, and geographic source.

MO095

Mass spectrometry-based analytical approaches to evaluate disinfection byproducts in chemically-disinfected water

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Water disinfection is essential to prevent the spread of waterborne infectious diseases. However, the use of chemical desinfectants results in the unintended formation of disinfection byproducts (DBPs). The type of DBPs that get formed depends on the organic and inorganic precursors present in the water (e.g., type of natural organic matter, iodide, bromide content) and the disinfection conditions (e.g., type of disinfectant, dose, contact time, temperature, water pH). Some of these compounds are highly cytotoxic and genotoxic to mammalian cells, according to *in vitro* assays. Moreover, exposure to DBPs have been associated to an increased incidence of bladder cancer and the apperance of negative effets at developmental and reproductive level in various epidemiological studies. The present study aims at characterizing the changes in the natural organic matter of different source waters used to produce drinking water after disinfection at plant scale, and investigating the disinfection byproducts formed. For this, target, suspect and non-target screening approaches using low, high and ultra-high mass resolution techniques have been used. *Acknowledgments:* CP acknowledges support from the Swedish University of Agricultural Sciences through the August T Larsson Guest Researcher Programme.This work was supported by the Government of Catalonia (Consolidated Research Groups 2017 SGR 01404-Water and Soil Quality Unit)

MO096

Development of an GC-QTOF suspect and non-target screening method for the detection of organic contaminants in waste water

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Domestic waste water must be managed effectively to meet the challenges of increasing population, stringent regulatory requirements, and aging water treatment facilities. To meet these challenges, specific analytical methods are available to monitor chemical compounds in wastewater. However, because of the complexity of the sample matrix, several analytical methods are required to determine polar and non-polar organic compounds in the dissolved and suspended phases that may impact water quality. To screen for such a large and diverse suite of compounds, it requires non-discriminating sample preparation techniques and, ideally, universal detection techniques. A screening method in the combination of ultrasonic extraction, gas chromatography- high resolution mass spectrometry detection and automated feature extraction was developed for suspect and non-target screening in the sewage influent and treated effluent from an municipal wastewater treatment plant. The samples were extracted by ultrasonication for 20 min using dichloromethane for four times. The samples were analyzed by full scan GC/Q-TOF with a mid-column backflushing configuration and a 20 minute retention time locked (RTL) method. The results were evaluated using MassHunter software and the Agilent GC/Q-TOF Pesticides & Environmental Pollutants PCDL library for suspect screening . The non-target screening was performed using deconvolution with identification of the cleaned high resolution spectra using the NIST 2017 library. The identified pollutants included the Environmental Protection Agency (EPA) priority pollutants, pharmaceuticals, herbicides, antioxidants, intermediates, organic solvents and chemical raw materials. The proposed method is proved to be a promising one for non-target screening of complex matrix samples with the advantages of higher sensitivity and better repeatability.

MO097

Analysis of micropollutants and metabolites in Hwapo wetland via target, suspect and non-target screening using LC-HRMS

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Hwapo, the biggest riverine wetland in Korea is flowing into the Nakdong river used as a drinking water source and is a habitat living about 800 species. However, the wetland is surrounded by agricultural, residential and industrial areas and thus is contaminated by various organic pollutants. This study is aimed to identify major organic contaminants in Hwapo wetland via target screening, and to tentatively identify their transformation products(TPs) via suspect and non-target screening using HPLC-HRMS. Water samples were collected from 5 inlet streams and 7 spots of main stream of the wetland in July and August, 2018. The collected samples were pretreated with SPE method using multi-layer cartridge to enrich diverse substances having different physic-chemical properties. As a result of target screening, 12 pharmaceuticals(caffeine, niflumic acid, etc.) and 5 pesticides (carbendazim, oxadiazon, etc.) were quantitatively identified in both periods, and 4 pesticides were additionally detected only in July. Among them, caffeine(July: 33~1100 ng/L, August: 56~580 ng/L), niflumic acid(July: 23~75 ng/L, August: 42~290 ng/L), carbendazim(July: 10~110 ng/L, August: 64~520 ng/L) and oxadiazon(July: 17~220 ng/L, August: 66~460 ng/L) were detected at a relatively high concentrations in most sites and were considered to be the major contaminants. Through suspect and non-target screening, 10 substances(tebuconazole, hexaconazole, tricyclazole, diuron, simetryn, fexofenadine, irbesartan, cimetidine, valsartan, and benzotriazole) and 4 transformation products or metabolites (TEB_M324c, azoxystrobin acid, 2-aminobenzimidazole, and valsartan acid) were tentatively detected. When the parent compound and its metabolites were detected together, the peak intensity ratio of TP to the parent was calculated as an index for rate of degradation in the wetland. rate of degradation was confirmed by [(metabolite peak intensity / parent compound peak intensity) * 100 (%)] equation. The ratio for carbendazim(July: 0.36~3.2, August: 0.13~3.6) and valsartan(July: 0.13~2.8, August: 0.9~8.1) were relatively higher than tebuconazole(July: 0.08~0.26, August: 0.03~0.18) and azoxystrobin(July: 0.27~2.1, August: 0.12~0.58), and showed an increasing trend with increasing hydraulic retention time in the wetland. This result indicates that less organic pollutants are discharging into Nakdong river due to degradation processes taking place in the wetland.

MO098

Mass spectrometry in combination with in vitro bioassays: a tool for minimizing the exposure to toxic disinfection byproducts

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Disinfection of water is a practice commonly used to ensure safety of drinking and reclaimed water. This practice results in the formation of a large suite of disinfection byproducts (DBPs) following the reaction of chemical disinfectants with organic and inorganic matter present in water. Many of these compounds have been reported to be highly genotoxic and cytotoxic in *in vitro* assays with Chinese hamster ovary (CHO) cells. Epidemiological studies have also associated their exposure with the appearance of negative effects at reproductive and developmental level, and even bladder cancer. Protection of public health calls for minimizing the formation of toxic DBPs in disinfected waters. This requires the development of analytical tools that allow identifying the most toxic DBPs in disinfected water so that appropriate measures can be adopted for minimizing them. The combination of mass spectrometry analysis and *in vitro* bioassays seems an appropriate tool to prioritize the study of toxic DBPs in disinfected water. Initial steps that should be taken in this direction are i) the evaluation of the toxicity response of the model cell line selected to individual DBPs and ii) the selection of a generic extraction protocol compatible with this bioassay that provides a representative DBP mixutre of the disinfected water. Due to the negative effects that DBPs may pose at developmental and reproductive levels, a human plancenta cell line (JEG-3) was selected to investigate their reprotoxic response. The generic extraction aproach will be selected evaluating the recovery of different DBP classes (iodinated trihalomethanes, trihalogenated haloacetaldehydes, halogenated acetonitriles, halogenated acetamides and haloacetic acids) with various extraction approaches (different SPE sorbents, liophylization). The present work summarizes the results obtained in these initial steps. *Acknowledgments:* C. Postigo acknowledges the financial support by Fundación General CSIC (Spanish ComFuturo Programme). This work was supported by the Government of Catalonia (Consolidated Research Groups 2017 SGR 01404- Water and Soil Quality Unit)

MO099

Effect-directed analysis of contaminants of emerging concern in hospital effluents

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The fate and distribution of new emerging contaminants and their unexpected effects in the environment are needed study topics, since most of the chemicals nowadays delivered in nature are unregulated and their effects are overlooked in many monitoring programs. These new chemicals include personal care products and pharmaceuticals (PCPPs) and consumption products (food additives, detergents, perfluoroalkyl substances, plasticizers, etc.). One of the main source points of those pollutants in aquatic ecosystems are wastewater treatment plants (WWTPs), which accumulate housing and industrial wastewaters and are not always able to degrade all these compounds. Then, they are delivered into rivers and marine waters with an unpredictable effect in the aquatic ecosystems. Within this context, an effect-directed analysis approach was implemented in the Galdakao Hospital WWTP effluents (Biscay, Spain). A large volume solid-phase extraction (LVSPE50) device was used on 35L of effluent water using a 6 g Chromabond HR-X (Macherey-Nagel), 2 g Sepra ZT-WAX (Phenomenex) and 2 g Sepra ZT-WCX (Phenomenex) sorbent combination. Afterwards, two fractionation steps were performed using C_{18} and aminopropyl chromatography columns. The fractions from the first separation step were tested with the available bioassays in order to look for the toxic fractions (i.e. in-vitro tests as YES/YAS and EROD). The identified as toxic were further fractionated and bio assayed a second time to restrict the identification of the toxic compounds. The recovery of the whole procedure (solid-phase extraction and fractionation) was assessed with a synthetic mixture containing over 200 standards. The non-targeted chemical analysis was run by liquid chromatography-high resolution mass spectrometer (LC-HRMS) using a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer with a heated ESI source (Thermo, CA, USA). The m/z values of the predicted compounds were searched in the peak list considering 5 ppm for mass tolerance and 30% for the intensity tolerance for the isotope search. Structural assignments were carried out based on $ddMS^2$ fragments annotated by Compound Discoverer 2.1 (Thermo-Fisher Scientific, CA, USA). Afterwards, we compared the exact mass, isotopic profile, fragmentation and abundances of the selected features with those available in the mzCloud (best match > 75 %) library.

MO100

Non-Target Characterization of Airborne Halogenated Organic Compounds by HPLC-HRMS

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A major mechanism by which persistent organic pollutants (POPs) become globally distributed is through long-range transport in the atmosphere. There are far more chemicals in commerce, and possible impurities and degradation products thereof, than are included in routine atmospheric monitoring. We hypothesized that unknown â_POP-like' halogenated contaminants are currently undergoing long-range atmospheric transport from today's major emission regions. With a large proportion of the world's population, a geographic shift of chemical production and use to China and India, and unregulated activities such as e-waste recycling by low temperature burning, Asia is an important source region that should be monitored by sensitive non-target analytical methods. The Maldives Climate Observatory at Hanimaadhoo (MCOH) is a large-footprint receptor for air transported long-range from regions of South Asia, including India and Bangladesh, thereby providing an opportunity for collection and nontarget analysis of persistent organic chemicals reaching this site in the Indian Ocean. A high volume air sampler was installed to collect fine airborne particulate matter (PM 2.5) on quartz fibre filters during 48-hour periods (700-1600 m^3) from January to April 2018 as part of the South Asian Pollution Experiment 2018 (SAPOEX-18) campaign. For some samples, semi-volatile organic compounds were simultaneously collected on downstream polyurethane foam plugs. Filters were sequentially extracted by sonication with a range of organic solvents to isolate a broad range of ionic, polar, and hydrophobic organic substances. Concentrated extracts were analysed by ultra-high performance liquid chromatography-high resolution Orbitrap mass spectrometry using data independent MS/MS. Methods for identifying halogenated features in the complex mass spectra were developed and implemented, based on isomer patterns of chlorine and bromine, characteristic fragment ions or neutral losses, and homologous series. Results show numerous features in PM 2.5 and PUF extracts with characteristic halogen signatures. For example, PM 2.5 contained a range of species with the general formula $C_7H_{6-n}X_nO_4$ (n = 1-2, X = Cl, Br, and/or I), and structural elucidation suggested these may be halogenated dihydroxy benzoic acids. New chemicals will be discussed with respect to their temporal variation throughout the campaign, and their regional sources by back trajectory analysis.

MO101

Human biomonitoring of a non-occupationally exposed population living near a hazardous waste incinerator: Temporal trends of PCDD/Fs in plasma

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In 2018, the occurrence of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in plasma from individuals living in the neighborhood of a hazardous waste incinerator (HWI) located in Constantí (Tarragona County, Spain) was assessed. Forty samples were collected from individuals of both genders, different age (19-69 years) and living in 2 areas of residence (urban and industrial).

Concentrations of PCDD/Fs were further compared to those already reported in the baseline study (1998), when the facility was still being built, and previous surveys (2002, 2007 and 2012), once the plant was already operative. In the current campaign (2018), the mean concentration of PCDD/Fs was 6.79 pg I-TEQ/g lipid, ranging from 0.23 to 32.7 pg I-TEF/g lipid. It meant a statistically significant reduction with respect to the baseline study (1998) as well as two previous campaigns (2002 and 2007), when mean PCDD/Fs levels in plasma were found to be 27.0, 15.7 and 9.4 pg I-TEQ/g lipid, respectively. However, current results were very similar to those previously reported in 2012 (6.18 pg I-TEQ/g lipid). When evaluating the PCDD/Fs congener profile, OCDD was the predominant congener, while 2,3,7,8-TCDD and 1,2,3,4,7,8,9-HpCDF presented the lowest levels. Anyhow, the individual concentration of most of the 17 PCDD/Fs congeners decreased over time. This reduction was actually reported in both genders, all group ages and residence areas, when considering the whole assessment period (1998-2018). In agreement with data from the scientific literature, a significant correlation between PCDD/Fs concentrations in plasma and age was observed. The current concentrations of PCDD/Fs in plasma of residents are in the lowest part of the range in comparison with results corresponding to other areas impacted by municipal or industrial solid waste incinerators. Hence, the human exposure to PCDD/Fs for the population living near the HWI located in Tarragona County does not mean any significant additional risk for the human health.

MO102

Investigation of metabolic changes in the freshwater amphipod Gammarus fossarum in response to sewage effluent exposure

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Chemicals coming from sewage effluents, can be detected in the aquatic environment in very low concentrations. Interest in exploiting “omics” approaches to screen the toxicity of chemicals in model species increased substantially in the last decade. Gammarids (*Gammarus fossarum*) have been shown as very sensitive to pollution. However, to date no reliable molecular markers are available in these species. The aim of this study is to investigate the changes in metabolome between amphipods sampled up- and downstream of an industrial wastewater treatment plant (WWTP). Differential metabolites can be used to further investigations of biological pathways affected by exposure to chemical mixtures coming from sewage effluents. *G. fossarum* were sampled from a Swiss freshwater stream in September 2017, using a standard kick-net method. Sampling was performed 50 m downstream and 50 m upstream of the WWTP. A total of 42 amphipods were sampled. A chloroform-methanol extraction protocol was performed on each amphipod and both polar and non-polar extracts were analysed using a LC-Orbitrap Elite platform. Each sample was analysed in both positive and negative ionization modes. Unsupervised multivariate models were built to calculate the rate of separation among the samples and univariate t-tests were performed to calculate the number of significant differential metabolites. Despite there were hints of separation between up- and downstream populations when looking at the PCA plots, for both male and female samples, t-tests showed no significant differential metabolites in female samples, neither in polar nor in non-polar extracts, in both positive and negative ionization modes ($p>0.05$). However, in the male polar extracts, t-tests revealed 28 and 69 differential metabolites, in negative and positive ionization modes, respectively. 44 differential metabolites between up- and downstream populations were detected in non-polar phase of the male samples, using a positive ionization mode. The source and identity of these metabolites is currently in progress. A further investigation of the differential metabolites found in the male samples could reveal impaired pathways in response to a chronic exposure to anthropogenic chemical mixtures in water. The metabolic fingerprints suggest different responses between female and male amphipods, supporting our transcriptomics data. These results suggest that males and females might be responding differently to waste water effluent.

MO103

Non-Target Analysis of the Organic Fraction of Fine Particulate Matter Using Gas Chromatography - High Resolution Mass Spectrometry

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Exposure to air pollutants and particles is associated with increased morbidity and mortality from cardiopulmonary diseases and lung cancer, and is estimated to cause 7 million premature deaths annually worldwide. Fine particulate matter (particles with an aerodynamic diameter < 2.5 Âm; $PM_{2.5}$) is one component of air pollution that has been suggested to play an important role for adverse health effects. The chemical composition of fine particulate matter is highly complex and heterogeneous, and depends on its primary sources and atmospheric transformation processes. Research suggests that the organic components of fine particulate matter have adverse effects on human health and the environment. Some of the adsorbed organic compounds, such as PAHs and quinones, are suggested to play an important role in the toxicity of particles. Other organics in $PM_{2.5}$, such as nitro-phenols, may also affect global climate because of their light absorbing properties, while adsorbed persistent organic pollutants may be subjected to long-range transport. However, much of the organic composition of fine particulate matter is still unknown and not routinely monitored. This work presents a non-target workflow for comprehensive chemical characterization of semi-volatile organic compounds in $PM_{2.5}$ by gas chromatography (GC) coupled with an Orbitrap high resolution mass spectrometer. $PM_{2.5}$ was collected at the Climate Observatory at Hanimaadhoo (MCOH) in the Maldives between January and April 2019 on quartz fibre filters using a high volume air sampler. MCOH is a receptor location for the Indian and Bangladeshi source regions. Filters were extracted by a range of organic solvents followed by pre-concentration and silica gel column cleanup. The organic components were separated by GC and ionized under different ionization modes for comparison: electron ionization at 70eV (EI), positive chemical ionization (PCI), and negative chemical ionization (NCI). Data were analyzed in various ways, including by: (1) spectral matching to NIST database in EI-mode, (2) assignment of unequivocal molecular formula in PCI-mode, followed by database searches in ChemSpider and MS/MS structural elucidation, (3) flagging of potentially persistent, bioaccumulative and toxic organohalogen compounds in NCI mode.

MO104

Soft ionization mediated by inert gas for screening of halogenated compounds by GCxGC-HRToFMS

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Recently, non-target screening by high resolution mass spectrometry (HRMS) coupled with gas or liquid chromatography (GC or LC, respectively) has been used to elucidate the myriad of environmental pollutants. The authors also have been developing a non-target screening method for environmental samples by using comprehensive two-dimensional GC (GC × GC) - high resolution time-of-flight mass spectrometry (HRToFMS) with electron ionization (EI). The EI method has several advantages, however, it is sometimes difficult to estimate molecular formula of unknown compounds when molecular ions are not detected. Therefore, we evaluated soft ionization mediated by inert gas aiming sensitive and selective detection of molecular ions for organohalogen compounds in environmental samples. GC × GC-HRToFMS analyses were performed with a Zoex KT2006 loop-type modulator (Zoex Corporation, Houston, TX, USA) installed on an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a JEOL AccuTOF GCv 4G (JEOL, Tokyo, Japan). Argon (Ar) or helium (He) was used as moderating gas for the soft ionization (negative ion mode) and compared to chemical ionization with methane (CH_4) in this study. A standard solution containing organohalogen compounds (ES-5521(CIL)) was measured at various parameters, such as reagent gas flow rate, ion source temperature and electron acceleration voltage. Optimum conditions were investigated based on mass spectra and intensities of molecular ions. As a result, the ion source temperature affected on sensitivity in soft ionization with inert gas (Ar or He), and lower temperature improved ionization rate of molecular ion especially for highly chlorinated compounds. As for PCB209, the highest intensity of molecular ion and less number of fragment ions were observed when Ar was used as moderation gas and ion source heater was off. After the optimization of parameters, a crude extract of house dust (NIST SRM 2585) was analyzed by GC × GC - EI/soft ionization - HRToFMS. The effectiveness of soft ionization mediated by inert gas for non-target screening will be discussed in the presentation. **<strong>Acknowledgments** The authors thank Mr. Yukinori Yahata of JEOL Ltd. for his support and technical comments. This work was supported by JSPS KAKENHI Grant Number JP17K12833, 17H00796.

MO105

Time trends of emerging contaminants in sediment cores from a wastewater impacted recipient identified by high resolution-mass spectrometry

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Sewage treatment plants (STPs) are a major source of emerging organic contaminants of concern to receiving waters, as conventional (three-stage) wastewater-treatment processes are insufficient for many of these compounds. In addition, degradation-processes lead to transformation products that add to the total number of chemicals in the environment. There has been a number of studies focusing on the target and non-target analysis of emerging organic contaminants in surface waters, but the knowledge about their distribution and fate in sediment is still scarce. In this study, suspect and non-target screening was performed in sediment cores from a wastewater recipient in a study area impacted by urban- and agricultural-influences. A reference sediment sample was collected before the STP and sediment cores were taken both in a transport- and an accumulation bottom downstream. An efficient extraction method using salting-out-assisted liquid-liquid-extraction (QuEChERS) was optimized to extract a large range of compounds. Target and non-target analysis was performed by using UHPLC coupled to an Orbitrap mass spectrometer operated in full scan MS mode with data-dependent acquisition (top N) MS/MS mode. Identification of unknowns was done by matching obtained mass spectra with spectral library data (mzCloud). A large number of tentatively identified compounds originate from pharmaceuticals and personal care products and show concentration trends with increasing sediment depth that are in agreement with results from our prior target findings. A majority of the identified compounds show increasing concentrations towards surface sediment, which agrees with an increased usage in recent years.

MO106

Automated solid-phase extraction for seven neonicotinoids and fipronil in river- and seawater samples.

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With the recent ban of some neonicotinoid insecticides and the increasingly strict regulations on the use of systemic pesticides, the demand for quick, reliable and efficient analytical methods with low quantification limits is increasing. While manual solid-phase extraction (SPE) is currently the method of choice regarding sample preparation and enrichment for the determination of neonicotinoid insecticides in aqueous samples, the drawbacks are an extensive workload and a vulnerability for process variations. Here, SPE-automation can be an appropriate approach to reduce the manual workload and thereby free up laboratory resources, while at the same time increasing process parameter stability. The goal of the work here presented is: 1) the optimization and automation of the SPE process, using a LC-Tech FREESTYLE XANA device (LCTech GmbH, Obertaufkirchen, Germany), for the HPLC-MS/MS determination of 7 neonicotinoids and fipronil in varying environmentally relevant concentrations, and 2) the method validation and subsequent application to real field samples collected in the Bohai Sea and selected rivers of the Shandong province (China). In order to achieve this goal, a comparative literature research laid the foundation upon which a suitable approach for the SPE of neonicotinoids and fipronil was selected, modified and optimized for automated sample clean-up of brackish and sea water matrices. Results on the recoveries and performance of the automated SPE, using hydrophilic-lipophilic balanced sorbent cartridges and common solvents, are reported.

MO107

An Online Solid Phase Extraction-High Performance Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantification of Neonicotinoid Pesticide Biomarkers in Human Urine

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Neonicotinoid insecticides are currently a widely used replacement for the toxic organophosphates and carbamates. They are also used in lawn treatments and are effective for flea control in cats and dogs. However, the extent of human exposure to neonicotinoids is limited. We developed a high-throughput online-solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry (HPLC-MS/MS) method to quantify the urinary concentrations of four neonicotinoid pesticides: acetamiprid, clothianidin, imidacloprid, and thiacloprid; and two neonicotinoid metabolites: N-desmethyl-acetamiprid and 5-hydroxy-imidacloprid. The method uses 0.2 mL of human urine and uses an overnight enzymatic hydrolysis of glucuronide-conjugated species. The six analytes of interest are retained, washed and concentrated via online solid phase extraction (SPE), separated from each other and other urinary biomolecules using reversed phase high performance liquid chromatography (RP-HPLC), ionized by heated electrospray ionization (HESI) in positive ion mode and detected using tandem mass spectrometry (MS/MS). Accuracy ranged from 91 to 116 % and precision ranged from 3.7 to 10 %RSD. Limits of detection are all $< 0.5\ \mu g/L$, depending on the biomarker. The method has adequate accuracy, precision, and sensitivity to be applied to large–scale epidemiological studies, such as the National Health and Nutrition Examination Survey.

MO108

Analysis of antibiotics in food producing animals by LC-MS/MS

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Antibiotics are used to treat bacterial infections of animals and human in the case of illness. Repeated use of same antibiotics, patients gets resistance to those bacteria. Drug resistant bacteria like MRSA, VERA are a worldwide problem to cure patients in case of infections. Many antibiotics are also fed to cattle and broiler chicken in the firms in Bangladesh like other parts of the world. People of Bangladesh feel that beef and broiler chicken meat are not safe for health due to residual antibiotics in the meat. To determine the residual level of tetracycline, oxytetracyclin and chlortetracycline, and six sulfa drugs namely, sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamerazine, sulfathiazole and sulfamethoxypyridiazine in beef and broiler chicken meat samples by LC-MS/MS coupled with ESI and QQQ mass analyzer, Shimadzu protocol of method of antibiotics analysis was modified and validated in terms of lope time, mobilizing gas flow rate, heating and collision energy. The modified method was validated by recovery experiments of control samples, spiking at 2 different concentration levels (5 & 10 ng/g). The accuracy was evaluated in terms of percentage recoveries and precision was estimated by determining the co-efficient variances. Sensitivity of the LC was measured by evaluating LOD and LOQ at ng/g level. Identification of certified standard tetracycline was done by Q1 and Q3 Scan of precursor ion and 3 product ions, m1, m2 & m3. Quantitation was done using calibration of product ions by MRM (Multi-reaction Monitoring). Intra- (n=5) and inter (n=3) day recoveries were found to be in the range of 86-104%. Residual antibiotics were found to be below the detection limit.

MO109

Distribution of organic micropollutants in water and sediments from Lake Mälaren, Sweden

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The occurrence and distribution of organic micropollutants (OMPs) were evaluated in lake water and sediment of Lake Mälaren (Sweden) in autumn 2017. A liquid chromatography-tandem mass spectrometry method was applied to determine the OMPs concentrations in water and sediment. The partitioning of contaminants between lake compartments was estimated by means of solid water distribution coefficients (K_d). A total, 32 and 29 OMPs were detected in lake water and sediment, respectively, at concentrations from low ng/L up to 89 ng/L (lamotrigine) in lake water and from low ng/g dry weight (dw) up to 28 ng/g dw (citalopram) in sediments. Carbamazepine, lamotrigine, caffeine and tolyltriazole were the most dominant compounds in lake water since they were found in all lake water samples collected. Eighteen OMPs were quantified in both matrices, which enable the calculation of K_d values. The highest concentrations of most OMPs were found in Lake Ekoln water and sediments, which is close to Uppsala, the fourth largest city in Sweden. This location is affected by WWTP effluent water from an incoming river and show that densely populated areas are more prone to adverse impacts by OMPs due to an elevated usage and discharge of OMPs to surrounding water bodies. Keywords: pharmaceuticals, aquatic environment, target analysis, sediments

MO110

Ethanol-blended fossil fuels: is reduction of atmospheric pollution the only concern?

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The Clean Air Act (42 U.S. Code § 7401) is one of the USA's most influential environmental laws. Under the Clean Air Act Amendments of 1990, oxygen-containing organic compounds such as methyl-tertiary-butyl ether (MTBE) or ethanol must be added to gasoline in some areas of the USA to reduce air pollution. Since then, the use of ethanol has increased, with many countries formulating various forms of ethanol-fuel mixtures. Blends of E10 or less are in use in more than 20 countries around the world. In the USA, ethanol represented 10% of the gasoline fuel supply in 2011. These initiatives are targeted at combating CO_2 and particulate emissions, as oxygenates leave behind less carbon footprints. Noble as it may appear, this innovation is not without attendant consequences. One major implication is the effect of co-solvency on the leaching potentials of petroleum hydrocarbons. Of these, BTEX (benzene, toluene, ethylbenzene, xylene) and PAHs (polycyclic aromatic hydrocarbons) are of greatest concern. This is due to their toxicity and persistence in the environment. For example, Snape et al. (2005) in a study of fuel spills in Antarctica showed that the relatively insoluble dimethylnaphthalenes were not degraded. Similarly, Wang et al. (1998) found increasing resistance to biodegradation with increasing alkylation in the naphthalene family. Compounding this problem is the fact that groundwater contamination by these compounds from above-ground and underground fuel spills has been of growing concern in recent years. This portends danger for humans owing to their carcinogenicity. In view of this, our research investigated the effect of ethanol addition on the leaching potentials of four groups of recalcitrant PAHs present in diesel fuel, namely, trimethylnaphthalenes, dimethylphenanthrenes, pyrene and methylpyrenes. Their movement (as components of diesel fuel) was followed in a lab-based study of 90 cm x ID 13 mm soil columns. In addition, the leachates from each column were examined using gas chromatography-mass spectrometry (GC-MS) to determine their molecular compositions. Our results reveal that ethanol addition enhances the leaching potentials of these otherwise hydrophobic contaminants; with 10% ethanol significantly eluting all PAHs studied. This highlight the need for energy scientists to carefully consider the environmental impacts of ethanol-blended innovations holistically. It is not enough to save the atmosphere and ruin the hydrosphere.

MO111
Challenges in complying with legislation; How far should one proceed to check whether the environmental quality standard is met?
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Within the framework of various international river basin programs organotin pollution is monitored in Dutch surface waters. Tributyltin (CAS number: 36643-28-4) is one of many chemical parameters that determine the chemical status according to the Water Framework Directive (WFD). Environmental quality standards for tributyltin are defined in the Priority Substances Directive (DIRECTIVE 2013/39/EU). Dutch water authorities have put great effort into readily measuring tributyltin concentrations in their surface waters. The poster presented at SETAC Europe 2019 will go into the developed analytical method, the challenges faced to comply with the so-called QA / QC directive, and results of measurements of tributyltin in the Dutch waterbodies as part of the second round of river basin management plans. The main challenge was to comply with the desired reporting limit of one third of the year average EQS, being 0.2 ng/L. For the first round of river basin management plans tributyltin could not be analysed on that level. This led to an unknown chemical status for tributyltin. Rijkswaterstaat has used a sophisticated method using GC-ICP-MS instead of GC-MS to determine tributyltin in surface water. Headspace solid phase micro extraction is used to collect analyte from the sample. Gas chromatography combined with inductively coupled plasma and isotope dilution sector field mass spectrometry is used to separate, identify and reliably quantify the analyte. Due to extensive automation the ultimate method is not at all time consuming abut took a considerable stretch of time to develop. The desired reporting limit was achieved. The monitoring program has ultimately led to a better insight into the chemical status due to tributyltin. It is now clear which waterbodies meet the environmental quality standards and which do not. This is important because this way mitigating measures can now be taken to improve the aquatic environmental conditions. The question however is how far one should go to make sure the desired reporting limit is reached. It can (and will) be argued that one should perform a cost-benefit analysis taking into account not only costs of sampling and analyses, but also predicted (future) costs of mitigating measures.

MO112
SETAC Global Chemistry Interest Group
G.P. Cobb, Baylor University / Department of Environmental Science
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Natural Resources in LCA: Extraction, Processing and Dissipation of Metals, Minerals and Plastics (P)

MO113
Assessment of the French metals demand induced by national consumption and its associated environmental footprint
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Two main characteristics of the circular economy (CE), as defined in the French strategy of energy transition towards a CE, are a resource efficient economy and a low carbon economy. To assess strategies to be implemented towards a CE, both of these characteristics have to be simultaneously assessed to avoid burden shifting. The aim of this work is to show how environmentally extended multiregional input output approaches (EEMRIO) can be used to do so. EEMRIO databases were developed to take into account the environmental impacts of international trades; they consist in the coupling of two tables respectively describing the industries interdependencies in a given region along with the interregional interdependencies and the environmental interventions related to each industry. In this work, the EEMRIO databases WIOD and EXIOBASE v3 are used to determine both the metal and carbon footprints due to metal extraction and production (namely the “metal carbon”) induced by the French domestic consumption. Given their characteristics and their mathematical handling, the EEMRIO allow to access different types of results: the metal footprint and the metal carbon content of the products and services consumed domestically by France and the metal footprint and the metal carbon content of the economic activities induced by French domestic consumption. Following these indicators with WIOD, from 1995 to 2009, the total metal footprint of the French domestic consumption raised from 101 megatons to 143 megatons while the metal carbon footprint decreased from 38.6 megatons CO_{2eq} to 32.9 megatons CO_{2eq}. Moreover, most CO_2 due to metal processing is emitted in France (the similar assessment performed with EXIOBASE v3 shows that this is due to iron and steel industries) while metals are no longer extracted in the French territory. As it is a bit more disaggregated, EXIOBASE v3 allows a more specific assessment of the metal sectors and its impacts, for example construction related products that contributes the most both to the metal footprint and to the metal carbon content. EEMRIO permits the environmental assessment of the consumption flux in a national or regional level. Here the assessment was made on metals, but studies on material and carbon footprints can also be made on other products or services. One of the main drawback of EEMRIO, that has to be kept in mind when assessing consumption scenarios, is the age of the data available in publicly available databases.

MO114
MARILCA: A new working group on Marine Impacts in Life Cycle Assessment
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Impacts from marine litter, including plastic litter, are currently not included in life cycle impact assessment. This means that while plastic bans are rapidly taking place, there is no methodology to support whether the alternatives are better choices or not, since potential environmental impacts cannot be compared on the same basis. A working group was launched at the end of 2018 under the UN Environment Life Cycle Initiative, and with Forum for Sustainability through Life Cycle Innovation (FSLCI) as a communication and network partner. The objective of the working group is to foster the harmonized development of environmental impact pathways and characterization factors for marine impact assessment in life cycle assessment (LCA), in priority associated with marine litter. The current, primary focus is plastic. This project will allow, in the short-term, to coordinate and encourage the harmonious development of impact assessment methods, and, in the longer term, to integrate the marine impacts in LCA, including those from plastic litter, in a consistent approach that is compatible with the LCIA framework. Three main phases will characterize the project: Phase 1: Provide a first Framework paper developing and illustrating the different impact pathways associated with marine litter to be developed and identify the gaps and building blocks (November 2018 – June 2019). Phase 2: Coordinate and launch different research aiming at filling identified gaps and act as a central scientific reference on the topic to avoid overlaps (2019 - 2022), welcoming members who are working and contributing on the topic. Findings and updates are regularly discussed with stakeholders via a platform and workshops. Phase 3: Consensus building (2023-2025). Deliver a harmonized and consensus-based impact pathway framework and methods addressing plastic litter impacts (and potentially other complementary marine impacts) in LCA. Updates on and plans of the working group members, projects and deliverable will be provided in this communication, with the intention to raise awareness of the community and interest towards this important work.

Measuring the Sustainability of Circular Economies: the Potential of LCA (P)

MO115
Life cycle environmental impacts of a novel value-creating method to co-process coal mine and electronic wastes
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The CEReS method is developed within a European research project aiming at value creation by co-processing coal mine and electronic waste. The processes developed involve using the acid mine drainage (AMD)-generating coal wastes in a way that a leaching solution (lixiviant) can be produced. This lixiviant can in turn be used to solubilise metals from printed circuit boards (PCBs) taken from electronic wastes (e-wastes). In this way, not only the environmental impacts from the two waste streams can be reduced but also valuable metals can be produced, which in turn can help avoid the mining for virgin materials. The aim of this paper is to present the life cycle environmental impacts of this novel method developed within the CEReS project. The functional unit is the processing of 1 tonne of PCBs and the system boundary includes their transportation, shredding and three core processes of the CEReS method: pyrolysis, bioleaching and char leaching. Data have been collected through mass flowsheets and a preliminary life cycle assessment (LCA) model has been created using the GaBi software and the GaBi professional and Ecoinvent databases. The preliminary model so far is not able to prove whether the CEReS method is beneficial because the data for the bioleaching and char leaching are not available yet and the exact amounts of the valuable materials that will be recovered remain unknown. When the model is finalised and the exact amounts of materials recovered are calculated, it is expected that the CEReS method will reduce environmental impacts compared to an alternative scenario where PCBs go to hazardous waste incineration. This will provide evidence that whether the CEReS method is environmentally beneficial as a Circular Economy approach to securing sustainable supply of critical raw materials.

MO116
Comparative environmental assessment of improved foam glass material using alternative waste glass streams (CRT and flat glass)
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Mechanical Engineering, Faculty of Sciences and Technology; J. König, Jozef Stefan Institute; F. Freire, ADAI/LAETA - University of Coimbra / ADAI-LAETA, Mechanical Engineering
Buildings' envelope plays an important role in the overall energy balance of buildings. Better thermal insulation is achieved with increased insulation thickness or with improved insulation performance. In order to meet the EU and global energy efficiency demanding requirements for net zero energy buildings, it is important to develop thermal insulation materials with even more superior thermal characteristics to reduce the wall thickness while increase its thermal resistance. The goal of this paper is to perform an environmental assessment of an improved foam glass using alternative waste glass streams as raw material as well as compare these materials with alternative thermal insulation materials available in the market (insulation cork board, EPS, XPS and stone wool). A cradle-to-gate life-cycle model was implemented for two options (CON13 and BG20) selected from a set of samples under experimental development to reflect a range of properties and thermal performance. CON13 uses CRT panel waste glass and BG20 uses waste flat glass.Two alternative functional units (FU) were assessed to increase the robustness of the analysis: 1 kg of material and 1 m^2 of insulation material providing 1 m^2K/W of thermal resistance (R). Presenting the results only per kg or cubic meter (as most LCA studies, including EPDs, present the results) may be limited and misleading as the primary function of an insulation material is to provide thermal resistance. The results for four environmental categories and non-renewable primary energy show that BG20 present lower environmental impacts (< 10%) than CON 13 when assuming 1 kg as FU while CON13 present lower environmental impacts when assuming 1 m^2K/W as FU. Both foam glass options present lower environmental impacts per kg than the alternative thermal insulation materials in three out of five categories analysed. For ozone layer depletion and non-renewable primary energy, stone wool present lower environmental impacts. It can be concluded that foam glass has a great potential for improvement in terms of environmental performance as present lower impacts than most of the conventional insulation materials. The use of a functional unit based on thermal properties proved to be a much more robust and holist unit when comparing insulation materials. Further research can be done on assessing the environmental benefits of including waste or recycled material on conventional insulation materials in a circular economy perspective.

MO117

Driving in circles: can car-sharing enable circular mobility? An investigation into the life-cycle impacts of car-sharing

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Car transport is inefficient in terms of fuel-use and material-use compared to public transport. These efficiencies can be improved by technology, but the car is unlikely to ever supersede public transport. Car-sharing is one possible solution to enable a more circular transport sector by inducing changes to both user behaviour and cars' use-intensity. Most impact studies suggest that car-sharing users get rid of a car when they join car-sharing; these users also often increase public transport use at the expense of car-use. Moreover, car-sharing providers are expected to respond to economic incentives to increase the longevity, fuel efficiency and use-intensity of their car fleets, providing further reductions to both emissions and resource use. However, car-sharing is a completely new way to "consume" mobility, and the full effects are uncertain. What is needed is a rigorous impact evaluation of car-sharing to assess what the true causal impacts of car-sharing are. In this study, we have completed a life-cycle analysis of car-sharing, including both user and provider responses, to estimate the impacts on both resources and the environment. The method is rooted in a systems approach and rigorous impact evaluation. First, we identified the mechanisms that affect environmental benefits. Second, we collected data from both car-sharing users and non-users to understand how car-sharing users' behaviour changes. Third, we created the life cycle inventory with the users' behavioural changes and data from car-sharing providers. Finally, an uncertainty and sensitivity analysis was performed to understand the effects of uncertainty, since car-sharing impacts are dependent on naturally variable and uncertain parameters. Additionally, the complex interaction of different effects means that, under certain circumstances, car-sharing may not lead to lower resource use or environmental benefits. In sum, this study demonstrates the advantages of the integration of impact evaluation techniques and sensitivity analyses with LCA to give a more holistic view on the impacts of car-sharing. The results of this study will offer guidance to policymakers and researchers who aim to maximise the benefits of car-sharing and minimise any negative effects.

MO118

Assessing circularity by addressing recovery efficiency, contribution and recyclability

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There is a growing need for assessing circularity. This has several reasons, such as to monitor the circular economy in relation to the policy goals set, or to compare products in order to choose the best. Another reason is that there is a need to prioritize those activities that will help society transition towards a circular economy. Currently there are several methods that aim to assess or measure circularity. The most basic being the classification following the 9R strategies or more complex following a Life Cycle Analysis approach. Here we present a method aimed at assessing circularity of material recovery options. Where recovery options are seen as any process that uses residual material or a waste stream for the production of a secondary material. The indicators presented here address the fact that resource efficiency has several pillars which need to be included in a circularity assessment: 1) efficiency of the materials recovery process, 2) the contribution of the secondary materials to the materials-market demand for such materials and 3) future recyclability (second loop recycling). Each of these indicators is based on the relevant material flows and expressed as a score between 0 and 1. The approach was tested using two case studies. First on recovery of the phosphate mineral struvite from waste water for use in artificial fertilizer and second on the recycling of rubber from end of life tires (ELT) for use as infill material in synthetic turf pitches. Struvite recovery scored between 0.09 and 0.47 on recovery efficiency, the uncertainty lies in taking into account the additional materials needed in the recovery process or not. The ELT rubber scores 0.9 in recovery efficiency. The contribution to phosphate use, is 1% for phosphate from struvite. The contribution to infill use in the Netherlands was almost 90%. The recyclability of struvite is based on the losses in the use phase as accounted for in a mass balance and results in a score of 0.16. The recyclability of ELT rubber from synthetic turf pitches is scored as 0. This method to assess circularity should inform different stakeholders in the material recovery processes, i.e. regulators for giving permits or industry for prioritizing R&D. Other topics are also relevant to address when permitting or prioritizing recycling options. For this reason the Safe and Sustainable Loops framework includes other methods addressing the environmental impact and the risks from chemicals or pathogens.

MO119

How sustainable is the functional economy? Literature review about sustainability assessment methods applied to functional economy.

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As shown by the multitude of scientific articles as well as policy plans about the circular economy (Kirchherr et al., 2017), scientists and policy makers realize the need to go from a linear resource consuming economy to a more circular economy. The circular economy (CE) is an economic model where environmental pressure is decoupled from economic growth. This model can be implemented by companies in different ways of cycling resources and with alternative business models. Five or six different categories of alternative business models have been identified in literature, one of those being the Product Service System (PSS), including product-oriented, use-oriented and result-oriented services. The two last subcategories are part of the functional (service) economy which is the branch of CE which we will analyze in our work. The functional economy (FE) can be simply defined as the sale of a use of services instead of a material good. We can find many claims in the literature that the FE is more sustainable than traditional good ownership-oriented business models. The main argument is that FE could increase the units of service provided per unit of resource used, and could incentivize towards high resource cycling rates, which would result in higher resource efficiency and lower environmental impacts. However, the research community, aware of the importance to assess if FE truly increases sustainability, acknowledges a lack of empirical evidence of these claims and mechanism, e.g. in the form case studies evaluating positive or negative effects of the new CE business models. Our literature review focus on different variants of PSS, their mechanisms to affect resource cycling, and tools to assess their potential of sustainability. Our review considers peer-reviewed articles presenting case studies and reports from companies about PSS. For each of them we will analyze how the authors assess the different dimensions of sustainability (environmental, economic and social). Furthermore, the quantitative occurrence of tools will be evaluated to have a better understanding on how scientists evaluate sustainability. The first results show that even though LCA is considered as very time-consuming to do for businesses (Manninen et al., 2018) they are still used a lot to analyze environmental aspects. We also find that companies often develop their own tools (Moesch et al., 2018), especially to link environmental with economic aspects.

MO120

The environmental behavior of PEEK as an innovative material in a new portable hydrogen fuel cell

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The E-Li-GE project (http://projects.ciemat.es/web/elige/home) funded by the Ministry of Science & Innovation of Spain, proposes a new way for portable energy generation, in the 1-100W power range. It is based on a hydrogen fuel cell (FC) with high power density. The key concept of the project is the integration of

the current collectors in the electrodes to decrease both weight and volume of the distribution plates and the end-plates, which largely contribute to reduce the FC power density. The new completely passive design also eliminates the weight and volume of unnecessary auxiliary systems and their associated power consumption, to make of this system a viable alternative for powering portable devices. In order to be compared to other alternative FC in terms of environmental aspects, the Environmental Footprint has been carried out. Polyether ether ketone (PEEK) is a thermoplastic with both excellent mechanical and chemical properties to high temperatures, which has been used as an innovative material due to its light weight and density. The objective of this work is to show the influence of the manufacturing of this material in the whole life-cycle of the FC.

MO121
Circular economy: Packaging recycling for energy savings and climate change mitigation
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Circular economy has emerged as a solution both for resources scarcity and dependency as well as for high environmental impacts related to the extraction of raw materials and waste management. High rate of consumption causes growing amounts of packaging waste - a specific waste stream, that gains a separate attention in EU waste policy. The most commonly used materials for packaging are paper, wood fibres, plastics, glass, steel and aluminium. Different packaging materials are associated with variety of environmental impacts during the whole life cycle of the packaging. Hence, recycling of those materials is one of the options for environmental pressure reduction. The aim of the study was to evaluate potential energy savings and greenhouse gas emission mitigation using recycled material instead of raw materials for beverage packaging. For this purpose, packaging use rate was evaluated in Lithuanian case. In the study, the primary energy consumption and GHG's indicator was estimated for 1 kg of packaging. Energy need and GHGs emission factors thru all life cycle of different packaging both from recycled and not recycled materials were selected from scientific literature review and applied for Lithuanian case. According to the packaging used, on the national level the largest primary energy consumption is for plastic packaging (~ 11 thousand GJ per year). If half of this type of packaging would be recycled around 3.000 GJ of energy could be saved. In the case of glass, the energy saving would reach 640 GJ, in the case of aluminium – even 4.000 GJ a year. In terms of GHG's in total about 37.000 tons of these emission could be saved with the aluminium packaging contributing the most into GHG's reduction. Hence, consumer awareness raising, and promotion of waste sorting behaviour still is important. In addition, the recycling of the packaging wastes could make a positive effect on the other environmental aspects, such as primary raw material and water use etc.

MO122
Is the single-use plastic ban by EU universally good? The case of airplane catering.
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Abstract The European Parliament has recently voted for a complete ban on single-use plastic cutlery, together with: plates, straws, drink-stirrers, etc. With this, there is a clear aim to stop plastic (both micro- and macro-) pollution of the oceans, caused by land and coastal littering. There are different ecodesign strategies, some of them compatible, others mutually exclusive, depending on the system the strategy is being applied to. The most frequent are: dematerialization, weight or energy reduction, recyclability, re-usability, durability, degradability, bio-based, etc. For instance, weight reduction and degradability are often mutually excluded with durability or re-usability; recyclability may be good for some simple products and not so good for complex ones. The EU has decided that the best (and only?) strategy for improving the environmental impacts of single-use plastic cutlery is a material change. Currently, most airlines are using single-use plastic cutlery or multi-use steel cutlery for their catering services. The Zero Cabin Waste project is studying the environmental impact of plane catering waste management. The Ceres project is studying food production and consumption strategies for climate change mitigation. It is known that more than 90% of the impacts of air travel occur during the flight, due to the energy needed for operation. Our findings show that, because of this fact, the most effective ecodesign strategy for airplane catering cutlery is weight reduction, which is not compatible (by an order of magnitude) with the shift from single use plastic to steel cutlery to avoid littering. A better alternative would be to ensure that the plastic items are 100% controlled, collected and recycled, which would avoid littering as well as other impacts such as climate change, acidification, eutrophication, resource depletion, etc. This is difficult to develop in the majority of cases but not within a so controlled system such as aviation. Other ecodesign alternatives are also explored within these projects. **Acknowledgement** The authors are responsible for the choice and presentation of information contained in this paper as well as for the opinions expressed therein, which are not necessarily those of UNESCO and do not commit this Organization. Zero Cabin Waste (LIFE/ENV/ES209) is co-financed by the EU through the LIFE Programme. Ceres-Procon (CTM2016-76176-C2-1-R) is co-financed by the Spanish Ministry of Economy and Competitiveness.

MO123
Enhanced adsorption of phosphate from wastewater using chitin-calcium based waste material: From nutrient recovery to a new fertilizer product
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Phosphorous (P) is an essential macroelement, but too much phosphate in aquatic ecosystems can lead to a deterioration in water quality and eutrophication. P recovery from wastewater streams, through the adsorption of P onto natural, solid waste material may provide one solution. Ideally, this would be a low-cost solution that would create a P-rich material (low in other adsorbed contaminants) for direct use in agriculture as a fertilizer. Crab carapace, a waste by-product from the seafood industry, is generated in millions of tons annually and large quantities are simply discarded as waste (i.e., to landfill). Therefore, a feasible approach may be valorisation of this solid waste into a high value-added product. Chitin-calcium based sorbent from crab carapace was modified thermochemically with KOH to improve surface chemistry and pore structure. The raw material was milled and sieved to a particle size of < 100 μm, mixed with a KOH solution, then the impregnated material (CCM) was heated. Low temperature activation showed good results, with a P removal efficiency up to 80 %. The maximum monolayer adsorption capacities (q_{max}) exhibited the following order: 42 > 32 > 22 °C with 24.85 > 23.88 > 21.36 mg/g, respectively. Instrumental techniques, such as BET analysis, scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDX), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and TGA/DTG analysis were used to determine the characteristics of the sorbent and future fertilizer material. The CCM had a high sorption capacity for phosphate, and low temperature activation was an innovative, sustainable and low-cost way to prepare a crab carapace-based sorbent for P recycling from wastewater. This research seeks to repurpose waste (crab carapace), close the P cycle and capture a globally limited resource (close the gap between P recovery and recycling), whilst also producing a new fertilizer product. Life cycle assessment (LCA) will be undertaken to help determine how best to create an efficient network of P capture (treatment) and re-use (as fertilizer) sites in rural locations. The sorbent will be tested in a FiltraPHOS™ pilot-scale P removal unit for use on small rural wastewater discharges. FiltraPHOS™ employs enhanced gravitational filtration through a granular media with continuous self-backwashing.

MO124
How critical raw materials could influence LCA impact categories in renewable energy systems
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Critical Raw Materials (CRM) awareness have arisen as both research and policy issues due to the future importance not only in economic but also in social and environmental world development. These concepts are related to those raw materials which are economically and strategically important for the world economy, have a high-risk associated with their supply, and there is a lack of viable substitutes. Energy systems, like PV solar modules or wind turbines, have metals or rare earths that are potentially included in these CRM. This work aims to identify the potential CRM in these technologies and quantify the potential influence on LCA impact categories, such as toxicity or abiotic resource depletion, when end-of-life actions to improve the sustainability and the circular economy, such as substitution or recycling of those materials, are carried out.

MO125
Life cycle assessment of hot-dip galvanizing process
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Hot dip galvanizing (HDG) is a surface treatment used to prevent steel corrosion. Zinc coating increases lifespan of steel in an economical way compared to industrial paintings. Although few studies have assessed the environmental impact of the whole production of galvanizing steel pieces, including steel production, none of them have analyzed the stages of HDG in detail. The aim of this work is

to conduct a life cycle assessment (LCA) of HDG based on data from this industry in Spain, within the framework of the EU project LIFE-2-ACID: *Towards a sustainable use of metal resources in the galvanic industry* (LIFE16 ENV/ES/000242). The cradle to gate study includes the extraction of raw materials, alkaline and acid degrease, acid pickling, flux, drying and hot dipping galvanizing, as well as waste and wastewater treatment. The life cycle inventory (LCI) referred to one ton of galvanizing steel was collected from Spanish companies by means of visits and questionnaires ensuring the quality and representativeness of the LCI, and for secondary data, Ecoinvent and PE International database were used. The system was modelled using Gabi software and CML 2001 including the categories of global warming potential (GWP) [kg CO_2 eq.], ozone depletion potential (ODP) [kg CFC-11 eq.], photochemical ozone formation (POF) [kg C_2H_4 eq.], acidification potential (AP) [kg SO_2 eq.], eutrophication potential (EP) [kg PO_4^{3-} eq.], abiotic resources depletion (ADP) [kg Sb eq] and abiotic-fossil resources depletion (ADP-fossil) [MJ]. The results showed that drying and hot dipping galvanizing stages have the highest contribution in most of categories, representing c.a. 90% of the impact in GWP, POF, AP, EP, ADP, and ADP-fossil because of the demand of zinc and the consumption of natural gas. In addition, the transport of raw materials had a significant impact in the GWP, EP, AP due to the emission of greenhouse gases and acid substances. Based on these results, LIFE-2-ACID project proposes a technological alternative to recover metallic zinc and iron chloride from spent pickling acids, which is the main waste generated in the HDG process. The innovative technological solution integrates membrane based selective extraction and electrowinning to recover up to 10% of zinc that is lost in the acid pickling. Thus, LIFE-2-ACID develops the emerging concept of circular economy using a hazardous residue as raw material available to reuse it in the same or other processes.

MO126

LCA on circular asphalt production in Denmark

H. Larsen, Danish Technological Institute / Research and Development

The technical possibilities and relative sustainability of using reclaimed asphalt in new wearing courses have been investigated in a recent Danish research project. The project focused on optimization of the individual steps of the process chain, starting from the milling of the old, worn asphalt pavement, to the production of the new hot asphalt containing recycled asphalt from worn out wearing courses. The necessity to mill off wearing courses and base courses separately, has been established. Following a comprehensive laboratory test program, full-scale validation of the concept was carried out on a part of a new highway in central Jutland of Denmark. All the obtained results unanimously show that – within the given frames – addition of 30% reclaimed asphalt wearing course into the production of new SMA wearing courses, as well as ABB binder courses, results in the same functional properties and expected durability as for 'virgin' produced reference asphalt material of the same type. In order to investigate the relative sustainability a comparative LCA study was performed comparing the new wearing and binder courses with conventional types. Results show overall better environmental performance of the new types with reclaimed asphalt, e.g. 14-22% lower carbon footprint. Including two different LCIA methods and combining with several sensitivity analyses confirmed the overall result.

MO127

Life cycle assessment of woody biomass ash in construction materials as a way towards a circular economy

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The forest biomass utilization as a way of mitigating climate change in the energy production sector is an excellent way but needs to be sustainable to ensure the environmental benefits. A fundamental issue that should be investigated when using forest biomass to produce energy relates to the ash management. The valorisation of woody biomass ash in construction materials appears to be a good management alternative. The purpose of this work is to compare the environmental impacts of different woody biomass ash valorisation alternatives using a life cycle assessment. The ashes under study include both fly and bottom ash generated from the combustion of eucalypt and maritime pine residues in a fluidised bed. The functional unit used in this study is the disposal/valorisation of 1t of woody biomass ashes. Four different management alternatives were assessed: cement mortar, concrete blocks, bituminous asphalt and disposal in landfill. The system boundaries include the transport of the woody biomass ash from the power plant to its destination, the on-site processes and the production of materials/energy consumed in each scenario. Furthermore, when the ash is valorised, the product system is credited with an avoided burden based on the reduced requirement for virgin material production (cement, sand, gravel and limestone). It was observed that the impacts of fly ash valorisation in cement mortar and concrete blocks presented differences of less than 2 %, indicating that the production of cement mortar and concrete blocks with fly ash have similar benefits, since it avoids the production of cement. For bottom ashes, the results show a negative net impact for all valorisation scenarios except for the production of concrete blocks, since the bottom ashes substitutes the gravel and its contribution is small and the substitution by ashes does not minimize the effects of this impact. It should be noted that the net benefits of bottom ash valorisation tend to be smaller than those obtained for fly ashes, as substitution is only possible for the aggregates and limestone. The bituminous asphalt production with bottom ashes substituting limestone presented the largest benefit for all impact categories, followed by the production of cement mortar production substituting sand. It was also observed that the valorisation scenarios have a better environmental performance than ash landfilling, resulting in a significant decrease of the environmental impacts in some scenarios.

MO128

Should electrical and electronic equipment be repaired for a second life? Environmental profile of electronic appliances: repair vs. substitution schemes

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Waste of electrical and electronic equipment (WEEE) is one of the fastest growing waste streams in Europe, expecting to grow up to more than 12 million tones by 2020. Given their complex mixture of materials and components —in some cases hazardous substances—, WEEE can cause environmental and health problems when they are not managed correctly. In addition to this, the production of modern electrical and electronic equipment (EEE) requires the use of scarce and expensive resources —i.e. critic raw materials— that sometimes are landfilled and not recovered. The released of the Directive 2012/19/EU established the base for WEEE stream management within EU countries, aiming at reducing environmental and health impact impacts, as well as increasing material recovery. Likewise, following the EU's Waste Framework Directive, polluter pays, extended producer responsibility and the waste management hierarchy principles were also incorporated into WEEE directive. As a result, the whole supply chain —from manufacturing to final disposal— of EEE must be addressed to comply with regulations and requirements. Achieving the established goals in terms of preparing for re-use and recycling implies different actions: increase separate collection, adapt or build a net of clean points in order to separate components and classify WEEEs, create a market for secondary raw materials and repaired appliances, among other. In this context, the preparation for re-use and recycling of EEE must be evaluated from a holistic approach. Life cycle assessment (LCA) allows analyzing the environmental burdens related to waste management and treatment taking into account the whole supply chain of electronic appliances. Hence, the current study evaluates recycling and re-using schemes of two EEE categories (i.e. Large Household Appliances (LHA) and IT equipment and telecommunications) throughout LCA. The results obtained shows that environmental performance highly depends on EEEs electricity. In this sense, despite the fact that EEEs life span is enlarged for a second life —avoiding the impacts derived from manufacturing and distribution stages— by means of repair or refurbishment, it might increase its electricity consumption when comparing to new appliances, which usually have a higher energy efficiency. Therefore, for some cases, the acquisition of a new appliance presents better environmental performance than a repaired/refurbished one.

Latest Developments and Future Needs for Higher-Tier Studies, Risk Assessment and Risk Management in the Regulation of Biocides, Pesticides and Pharmaceuticals (P)

MO129

Regulatory ERA of Veterinary Medicines may be improved: I - Need for other and better exposure scenarios for surface water

I. de la Casa Resino, Spanish Medicines Agency / Veterinare Medicines; C. Moermond, RIVM / Centre for Safety of Substances and Products; A. Haro Castuera, C. Rubio Montejano, G. Cortés Ruíz, Spanish Medicines Agency / Department of Veterinary Medicines; R. Carapeto-García, Spanish Medicines Agency / Veterinary medicines

The main source of emission of many Veterinary Medicinal Products (VMPs) into the environment is the use of manure as fertilizer. In the current Environmental Risk Assessment (ERA) of VMPs, $PEC_{SURFACE\ WATER}$ is calculated running the FOCUS model, using a winter cereal scenario. However, the input data recommended by the relevant VMP ERA guidelines are not fully detailed. This leads to inconsistencies in the predictions. We propose an improved parametrization of FOCUS model for winter cereals. Although it is acknowledged in the ERA guideline that manure is often spread on grassland, this option is not considered for the determination of $PEC_{SURFACE\ WATER}$. The lack of a grassland scenario may affect the accuracy of predictions. Thus, it may be necessary to implement this scenario. The winter cereal scenario does not necessarily pose a worst case situation, that also covers a grassland application. In a crop scenario the VMP will be mixed in soil during ploughing while in grassland this dilution is negligible. Consequently, direct runoff is likely to be quantitatively more important in grasslands. Besides, for the winter cereal scenario it is assumed that manure will be spread once a year, while in grasslands it can be spread several times per year (both with a maximum kg N per acre per year). Thus, by including the grassland scenario in the current ERA of VMPs the conclusions would be more robust. There are some limitations for the implementation of the grassland scenario: 1) **Grassland scenario is not defined in FOCUS:** We reflect on this issue in another poster. 2) **Diversity of European agriculture:** Grasslands are dominantly present over the crops in certain countries while in other countries grasslands may be irrelevant. The differences could be so wide that it would be worthwhile to consider a zonal-RA. Regarding the winter cereal modelling, in the VMP ERA guideline a number of default input parameters regarding the FOCUS PRZM model is lacking. For instance, there is no recommendation regarding the Chemical Application Method and depth. Depending on the input parameters the results of the model can vary substantially. We propose the use of homogenous mixture of VMP during ploughing ('CAM 4') and a depth of 20 cm for the runoff scenarios (20 cm is an assumed ploughing depth in arable crops).

MO130

Regulatory ERA of Veterinary Medicines may be improved: II - a grassland scenario proposal

I. de la Casa Resino, Spanish Medicines Agency / Veterinare Medicines; C. Moermond, RIVM / Centre for Safety of Substances and Products; A. Haro Castuera, C. Rubio Montejano, G. Cortés Ruíz, Spanish Medicines Agency / Department of Veterinary Medicines; R. Carapeto-García, Spanish Medicines Agency / Veterinary medicines

The main source of emission of many Veterinary Medicinal Products (VMPs) into the environment is the use of manure as fertilizer. The current Environmental Risk Assessment (ERA) uses a modelling approach (FOCUS) to estimate the predicted surface water concentration ($PEC_{SURFACE\ WATER}$). In this model it is assumed that application of manures coincides with drilling of winter cereals. A grassland scenario is not available (See Poster I), although it is assumed to be more relevant to VMP applications to soil. Although in some countries manure is mainly spread on and mixed into arable land used for crop production, in other countries it is common practice to distribute manure directly onto grassland. Therefore, we aim to make a first proposal for the parameters and crop grouping that could be taken into account to develop a realistic grassland scenario for use in FOCUS models. Besides the currently used scenario for *winter cereals*, a *grass/alfalfa* crop is also defined in FOCUS. This scenario could be used as a pragmatic surrogate for grassland. The periods of manure application in grassland are usually between the 1st of February to the 1st of September. These applications dates are out of the application dates stablished in FOCUS for *winter cereals* (25th September to 15th of July) and therefore, do not cover all possible grassland applications in a realistic way. The applications dates considered for the grass/alfalfa crop cover the whole year and therefore manure application dates (1st February to 1st September) could be adjusted in a more realistic way. Furthermore, 3-4 applications per year may be applied in grassland. The mixing depth with the soil depends on the manure application system. Typical method is the surface application of manure in grassland through broadcast spreaders. In this case a mixing depth of 5 cm can be applied. Please note that the feasibility of the grassland scenario to be used in the marketing authorization process should be intensively discussed in a regulatory context before being used.

MO131

Development of a test method for transformation of veterinary pharmaceuticals and biocides in liquid manure

T. Junker, ECT Oekotoxikologie GmbH; S. Berkner, German Environment Agency / Section IV 2.2 Pharmaceuticals, Washing and Cleansing Agents and Nanomaterials; R. Duering, Justus Liebig University Giessen / Institute of Soil Science and Soil Conservation; S. Fiebig, Noack Laboratorien GmbH; D. Hennecke, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecological chemistry; M. Herrchen, Fraunhofer IME / Ecological chemistry; S. Konradi, Federal Environment Agency (Umweltbundesamt) / Section IV 2.2 Pharmaceuticals; M. Meinerling, IBACON GmbH; U. Merrettig-Bruns, Fraunhofer Institute for Environmental Safety and Energy Technology UMSICHT; S. Thiele-Bruhn, University of Trier / Soil Science; E. Topp, Agriculture and Agri-Food Canada (AAFC); W. Völkel, Innovative Environmental Services IES Ltd / Environmental Fate; J. Roembke, ECT Oekotoxikologie GmbH

Spreading of manure on agricultural soils represents an important path by which veterinary pharmaceuticals and biocides enter the environment. To assess their environmental impact in the context of authorization processes, experimental testing of transformation of these compounds in manure is considered in regulatory guidance documents. However, there is no standardized experimental test method available so far. To fill this gap, a reliable experimental test method was developed to examine transformation of veterinary pharmaceuticals and biocides in liquid cattle and pig manure. The development was a step-by-step procedure. In the first project, preliminary studies were performed with regard to manure characterization, sampling and storage. Manure parameters to comprehensively describe and characterize the respective manure were identified and their variability due to differences in storage conditions and in manure origin was analyzed. A standardized procedure was established to homogenize manure both in the manure storage tank and in the laboratory prior to performing transformation studies. In addition, the influence of manure storage conditions (duration and temperature) and manure acclimation periods in the laboratory on the microbial activity of manure was tested. Subsequently, two anaerobic intra-laboratory test series with ^{14}C-labelled compounds were performed to examine the influence of test set-up (flow-through vs. semi-static, flow rate, amount of manure) on mineralization rates and the influence of type and origin of manure on transformation rates (UBA Texte 78/2015, UBA Texte 78/2016). In a follow-up project the reproducibility of the test method was proven in an international validation ring test (UBA Texte 80/2016). The results and experience gained in both projects, as well as scientific discussions at two technical workshops and several international meetings, where then put together in a draft test guideline on anaerobic transformation in liquid manure. This draft contains a technical description of sampling, manure characterization, test design and chemical analysis, as well as recommendations to identify transformation products and the definition of quality criteria. The draft test protocol is planned to be submitted for evaluation and approval as standard test method by the OECD.

MO132

Veterinary pharmaceuticals and natural hormones in the environment after field applications of slurry

J. Lahr, Wageningen Environmental Research / Team Animal Ecology; A. Derksen, AD eco advies; L. Wipfler, Wageningen Environmental Research / Environmental Risk Assessment Team; M. van de Schans, B. Berendsen, M. Blokland, RIKILT; W. Dimmers, P. Bolhuis, R. Smidt, Wageningen Environmental Research

The Netherlands are a country of intensive livestock rearing. It is well known that the livestock sector uses significant quantities of veterinary pharmaceuticals (VMP's) such as antimicrobials, parasiticides, coccidiostats and various other groups of substances. In addition livestock is emitting natural hormones. In order to study the environmental fate of these groups of compounds, a field survey was conducted in the province of Gelderland in The Netherlands. Five pig rearing and five veal calve rearing farms situated on sandy soils were selected. In the early spring of 2017, the slurry collected over the winter season at these facilities was injected into arable fields and grasslands. This is common practice in The Netherlands in order to prevent ammonia emissions into the air. During four months after injection, the concentrations of VMP's and hormones were monitored in soil, in the shallow ground water under the fields and in the surface water and sediment of adjacent ditches. Most VMP's of which the farmers reported their use were detected in the slurry from the pits before land application. These included various tetracyclines, sulfonamides, macrolides and quinolones. The slurries also contained a large number of estrogens, androgens, progestagens and their metabolites. Several more hydrophobic substances were found to persist to various degrees in soil after application: the antimicrobials oxytetracyclin, flumequin and tilmicosin (the latter two are typical of veal calve rearing and possibly persisting for over a year in soil), the parasiticide flubendazole, the coccidiostat toltrazuril(-sulfon) and the androgenic hormones androstendione and β-testosterone. As expected, soluble VMP's tended to be found more often in ground and/or surface water: the antimicrobials sulfadimidin (synonym of sulfamethazin), sulfadiazin and tiamulin (but few hormones). In five regional streams in the same region, sampled once in July of the same year, some of the more soluble VMP's and hormones were also detected. The pregnancy hormone progesterone was omnipresent at low concentrations in all environmental matrices but not in any slurry samples. Its source is therefore unknown. The presence of the mentioned compounds in the environment as a consequence of slurry application warrants a more in depth assessment of the risks for soil life, aquatic organisms and the consequences for the production of safe drinking water from ground water.

MO133

Environmental risk assessment of veterinary pharmaceuticals - lessons learned from terrestrial effect data

S. Schwarz, German Environment Agency UBA; J. Bachmann, German Environment Agency UBA / Section IV2.2 Environmental Risk Assessment of Pharmaceuticals; J. Brueckner, W. Koch, Umweltbundesamt / German Environment Agency / Section IV Environmental Risk Assessment of Pharmaceuticals; G. Speichert, German Environment Agency UBA

Since the guidelines on the environmental impact assessment for veterinary medicinal products (CVMP/VICH/592/1998 & 790/2003) came into force, the German Environment Agency (UBA) is tasked with the environmental risk assessment of veterinary pharmaceuticals. Applicants seeking approval of medicinal products follow the Phase I guideline, which, if necessary, includes calculating predicted environmental concentrations (PEC). In case the PEC exceeds 100 μg/kg in soil, or if the substance is a parasiticide used on pasture animals, fate and effect data have to be provided in the more detailed Phase II assesment. Terrestrial effect studies include the investigation of plant growth (OECD 208) and earthworm reproduction (OECD 222). Furthermore, effects on development of dung flies and beetles (OECD 228 and OECD GD 122) are considered in the case of parasiticides used on pasture animals. Both the applicant and the assessor evaluate the studies to assure adequate data quality. The effect data are used to derive predicted no effect concentrations (PNEC) for the respective substance. Over the last decade, this regulatory work resulted in a comprehensive data base containing effect data on active pharmaceutical ingredients (APIs) – which was evaluated in our current project: The vast majority of APIs used in veterinary medicinal products belong to the groups of antibiotics and antiparasitics, which include a variety of highly potent substances. Approximately 40% of PNECs derived for soil organisms are below 100 μg/kg, illustrating that the current PEC action limit is not protective enough for the soil compartment. Furthermore, dung organisms frequently prove to be highly susceptible to antiparasitics used on pasture animals, which necessitates adequate risk mitigation measures for these substances. Our results will help to identify possibilities and limitations of the current regulatory approach, and provide information for future modifications of the regulatory framework.

MO134

Revison of the guideline on environmental risk assessment of human pharmaceutical products. Part I: Outline, data collection and tailored strategies.

C. Moermond, RIVM / Centre for Safety of Substances and Products; S. Brendler-Schwaab, Federal Institute for Drugs and Medical Devices; E. Griffin, Health Products Regulatory Authority; J. Jensen, Aarhus University / Department of Bioscience; B. Scholz, Swedish Medical Products Agency; L.S. Nilssen, The Norwegian Medicines Agency; H. Stemplewski, Medicines and Healthcare products Regulatory Agency; R. Whomsley, European Medicines Agency; I. Rönnefahrt, German Environment Agency - UBA / Section IV 2.2 Pharmaceuticals

According to Art. 8(3) of the European Directive 2001/83/EC an environmental risk assessment (ERA) is required for all new marketing authorisation applications for a medicinal product. This ERA is based on the use of the product and the physico-chemical, ecotoxicological, and fate properties of its active substance. A draft of the revised ERA guideline has been published for consultation by the European Medicines Agency in December 2018. This guideline describes how to perform this ERA and how to evaluate potential risks to the environment arising from the use of the medicinal product, with the aim of protecting aquatic and terrestrial ecosystems including surface water, groundwater, sediment, soil and secondary poisoning - and the microbial community in sewage treatment plants. Furthermore, the identification of potential hazards (PBT; Persistence, Bioaccumulation and Toxicity) of the active substance is described. In a series of 3 posters we will present this revised guideline, with a focus on the main changes. \n Poster I focuses on the general outline of the risk assessment. Information requirements, data quality, and the way data is collected and evaluated will bediscussed. Information is given on the tailored assessment strategies for antimicrobial substances and endocrine active substances and on the use of the total residue approach. In this poster, also the PBT assessment will be discussed. \n

MO135

Revison of the guideline on environmental risk assessment of human pharmaceutical products. Part II: Phase I risk assessment and PBT assessment.

C. Moermond, RIVM / Centre for Safety of Substances and Products; S. Brendler-Schwaab, Federal Institute for Drugs and Medical Devices; E. Griffin, Health Products Regulatory Authority; J. Jensen, Aarhus University / Department of Bioscience; B. Scholz, Swedish Medical Products Agency; L.S. Nilssen, The Norwegian Medicines Agency; H. Stemplewski, Medicines and Healthcare products Regulatory Agency; R. Whomsley, European Medicines Agency; I. Rönnefahrt, German Environment Agency - UBA / Section IV 2.2 Pharmaceuticals

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MO136

Revison of the guideline on environmental risk assessment of human pharmaceutical products. Part III: Phase II risk assessment.

I. Rönnefahrt, German Environment Agency - UBA / Section IV 2.2 Pharmaceuticals; S. Brendler-Schwaab, Federal Institute for Drugs and Medical Devices; E. Griffin, Health Products Regulatory Authority; J. Jensen, Aarhus University / Department of Bioscience; B. Scholz, Swedish Medical Products Agency; L.S. Nilssen, The Norwegian Medicines Agency; H. Stemplewski, Medicines and Healthcare products Regulatory Agency; R. Whomsley, European Medicines Agency; C. Moermond, RIVM / Centre for Safety of Substances and Products

According to Art. 8(3) of the European Directive 2001/83/EC an environmental risk assessment (ERA) is required for all new marketing authorisation applications for a medicinal product. This ERA is based on the use of the product and the physico-chemical, ecotoxicological, and fate properties of its active substance. A draft of the revised ERA guideline has been published for consultation by the European Medicines Agency in December 2018. This guideline describes how to perform this ERA and how to evaluate potential risks to the environment arising from the use of the medicinal product, with the aim of protecting aquatic and terrestrial ecosystems including surface water, groundwater, sediment, soil and secondary poisoning - and the microbial community in sewage treatment plants. Furthermore, the identification of potential hazards (PBT; Persistence, Bioaccumulation and Toxicity) of the active substance is described. In a series of 3 posters we will present this revised guideline, with a focus on the main changes. \n Poster III shows the phase II risk assessment, for which it is mandatory to perform fate and ecotoxicity studies. Test requirements for the risk assessment of surface water, sediment, and sewage treatment plants are discussed. Depending on trigger values (see poster II), also a risk assessment for soil, groundwater and/or secondary poisoning needs to be performed. In this poster the way the risk assessment is performed for all these compartments is presented. Particular attention is given to the changes in the risk assessment strategy of the revised guideline compared to the ERA guideline currently in force (EMA/CHMP/SWP/4447/00 corr 2). \n

MO137

Recommendations for a future risk assessment tool for antimicrobial resistances in the environment. A regulatory approach.

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Therapeutic failure due to antibiotic Antimicrobial Resistance (AMR) is expected to cause more deaths than cancer by 2050. Action has been taken tackling the problem from a multidisciplinary approach following a "One health" philosophy. Environment plays a key role in the appearance and spread of AMR because it is a link between microorganisms in animals and people and it is a scenario for transmissible transmission of genetic variability and eventually receives antimicrobials as emissions from different sources (excreta, natural producer biota, etc.). Monitoring data on resistances in the environment, as already occurs for human and animal health, could be extremely useful for linking resistances to emissions and provide data to explore other factors that might affect to the appearance and spread of resistances. If risk assessors could reasonably predict risk for a resistance to arise and ultimately cause therapeutic failure, this would be a very much appreciated weapon to contribute to antibiotic prudent use. Assessors need that this could serve as a harmonized systematic decision tool. As it has happened in other frameworks, a "learn by doing" motto should be applied here, since prompt action is needed. The system should stablish a decision tree for the regulators to find systematic recommendations in order to refine risk. The workflow would proceed through a tiered strategy, with a simple, risk discarding approach as starters and proceed to a more refined analysis where experienced guidance by Academy or researchers should be sought by assessors. A battery of feasible risk mitigation measures need to be available. Prescribers and consumers should be well informed of the mitigation options and how to proceed for the measures to become a reality. The approach might need to consider a "think global, act local" horizon.

MO138

Can eprinomectin, a veterinary drug, induce chromosomal and nuclear damage at environmental concentrations?

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In order to maintain the high productivity of the livestock sector and reduce financial losses caused by different diseases, large quantities of veterinary drugs are used by rural producers. Eprinomectin is one of the most commercialized drugs for this purpose and it is found in soil in concentrations ranging from ng/g to µg/g. It belongs to the avermectin family, commonly used to combat a wide variety of parasites, and approximately 90% of the drug can be excreted in the dung of treated animals, contaminating the soil. So the objective of this study was analyzing the ability of environmental concentrations of eprinomectin to cause damage in the genetic material of *Allium cepa*, widely used as a plant bioindicator. Fifity seeds of *A. cepa* were exposed in Petri dishes containing soil contaminated with eprinomectin in different concentrations: 0.5 µg/g, 2.5 µg/g, 12.5 µg/g and 62.5 µg/g. Negative control was performed with soil+distilled water and positive control with soil+herbicide trifluralin. As the stock solution to contaminate the soil with eprinomectin was prepared with ethanol, a control containing soil+ethanol was also performed. Tests were conducted in triplicate in a BOD-incubator at 23°C and 12:12 light/dark. After five days of exposure, 45 slides per treatment were made with root cells from the meristematic region; 300 cells per slide were analyzed through a microscope in order to assess the induction of micronuclei and chromosomal/nuclear alterations (chromosomal bridges and loss, C-metaphase, nuclear bud, etc). Normality and homogeneity of data were assessed by Shapiro-Wilk's and Levene's tests, respectively. Afterward, the data were analyzed by Kruskal-Wallis/Dunn statistical test. The statistical test showed that the highest concentrations of eprinomectin (2.5 µg/g, 12.5 µg/g and 62.5 µg/g) induced statistically significant micronuclei formation and 12.5 µg/g concentration induced chromosomal/nuclear alterations in a significant way. Micronuclei presence is considered a biomarker of genotoxic and chromosomal instability events, which, if not repaired, indicate mutagenic effect. Chromosomal aberrations are characterized both by changes in chromosome structure and in its total number and are also used as a genotoxic biomarker. According to the results, environmental concentrations of eprinomectin can damage the genetic material of *A. cepa*, mainly by means of micronuclei formation in highest concentrations.
Financial Support: FAPESP 17/26214-8

MO139

Developing methodologies to understand the degradation and adsorption of macromolecule biopesticides in soil

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Pest control represents the main tool for enhancement of agricultural production. Since 1950s, with usage of chemicals (i.e. DDTs and organophosphates), agricultural yields improved to the detriment of environmental quality. To date, conventional pesticides continue to represent a risk for human/animal health and ecological biodiversity because of their environmental persistence and broad-spectrum action. Thus, the development of novel biopesticide which are less harmful, more selective, and thus more eco-friendly, represent a valid alternative in reducing these drawbacks. Therefore, we need to fully assess all new active substances. Global interest in these new pesticides is growing. In the last few years, the sector grew from 3.5% to 5%, acquiring some market share of conventional pesticide. Therefore, one big challenge in agricultural and environmental science is to achieve better knowledge of the environmental fate of novel biopesticides, for example by determining their behaviour in soil. Double stranded RNA (dsRNA) is a biomolecule that triggers the mechanism of RNA interference (RNAi). dsRNA is a protein disruptor, in eukaryotic cells, and it has considerable potential as a tool for insect control. Procedures for its extraction, purification and quantification from soil are essential for environmental fate assessments. We systematically developed a soil extraction procedure recovering roughly 80% of dsRNA from loamy sand soil. During adsorption experiments following the OECD 106 Guidelines for Testing of Chemicals, we found that pre-equilibrating the soil with a low concentration of CaCl2 solution (0.01M) resulted in precipitation of dsRNA (CaCl2 solution + dsRNA), which it might neutralize the charges on the phosphate backbone. This enhanced adsorption as compared to equilibration with distilled water. \n\n

MO140

Emergence timing and voltinism of phantom midges, Chaoborus spp., in the UK.

R. Cockroft, C. Gamblin, AgroChemex Environmental Ltd

The emergence timing of adult *Chaoborus* spp. (Diptera: Chaoboridae) was investigated in outdoor freshwater microcosms in the UK in 2017. Adult *Chaoborus* spp. started emerging on 13 April, 14 days after larvae had been introduced, and reached a peak on 2 May, 33 days after introduction of the larvae. The majority of emergence was completed by 3 June (65 days after establishment). Of the 2027 larvae introduced into four microcosms, emergence ranged from 51.4% to 66.2% with a mean of 60.9%. The majority of emerged insects were *C. obscuripes* (99.68%) and the remainder of those identified were *C. crystallinus* (0.24%). Males appeared to emerge slightly earlier than females. The number of *Chaoborus* spp. life-cycles per year occurring in the UK was studied in six replicate microcosm groups, each containing four individual units. Populations of zooplankton and benthic invertebrates were established in each mesh-enclosed microcosm (containing 200 L of water) with alder (*Alnus glutinosa*) leaves added to provide a substrate for benthic invertebrates. As soon as the first egg rafts were laid in each microcosm these were removed and introduced onto a fresh unit. The first generations in both replicate groups contained both *C. obscuripes* and *C. crystallinus*, although the larvae sampled from the second to fourth generations in these replicates were all *C. crystallinus*. Since only *C. crystallinus* was found in these later generations, the egg-to-egg development time for *C. obscuripes* could not be determined. For *C. crystallinus,* life cycle times from egg-to-egg ranged from 14 days (replicate group 5, first generation) to 56 days (replicate 3, second generation). The results of this study showed that *C. crystallinus* produced up to four discrete generations within the experimental period. The conditions existing in the experimental microcosms may have been unfavourable for oviposition by *C. obscuripes*. This study confirmed that in temperate conditions *C. crystallinus* exhibits a multi-voltine life history.

MO141

Roadmap to recovery: the value of the ecological recovery option (ERO) in higher tier aquatic studies

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The 2013 EFSA guidance document on tiered risk assessment for plant protection products (PPPs) for aquatic organisms in edge-of-field surface waters allows for regulatory acceptable concentrations (RACs) to be derived using two options. The ecological threshold option (ETO), which allows for negligible population effects; and the ecological recovery option (ERO), where some population-level effects occur, but recovery is demonstrated within an accepted time period. Under the tiered effect assessment, all tiers (tier 1-3) can address the ETO, however, only higher tier studies (tier 3) can be used to derive an ERO. The guidance states that it is feasible to determine an ERO if all issues regarding recovery have been addressed, with all relevant processes that determine population viability and the propagation of effects to the community, ecosystem and landscape level have been considered. However, the use of the ERO endpoint is often member state specific, and the current opinion of the Central Zone (CZ) Ecotoxicology Harmonisation Group is only the ETO approach should be used. Whilst this is certainly the more conservative approach when assigning endpoints and determining RACs, the application of a justifiable ERO-RAC should not be discounted in all cases. One of the key concerns of the CZ is the effect of multiple exposures on the ability of aquatic organisms to recover. Such exposures could occur from multiple inputs from the target crop (e.g. drainflow events), or by exposure to other products used within the same catchment. Since the EFSA guidance proposes an 8-week recovery period, it is possible that multiple exposures may occur during this time. However, the restrictive ETO only approach means that the ERO-RAC is not presented even when recovery is demonstrated to be rapid and the upstream use of compounds with a similar mode of action within that recovery period is unlikely. This poster builds a roadmap of the considerations that should be taken into account when proposing an ERO-RAC for regulatory purposes. This includes the experimental study design (frequent sampling to enable rapid recovery to be demonstrated), analysis of the crops grown and associated product usage within typical agricultural landscapes/catchments and the potential for recolonization of affected water bodies. A major objective of this poster is to generate an active discussion around the use of recovery as an endpoint across industry, policy makers and regulators.

MO142

Robustness of PT values based on the number of tracked animals

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For the risk assessment for wild mammals the proportion of an animal's daily diet obtained in habitat treated with pesticide (PT) is frequently used to refine the exposure. The PT values are typically obtained from field studies in which mammals are radio-tracked in the crop of interest and its surroundings. In most studies a rather small number of animals is tracked. However, the habitat use of individual animals may differ from the habitat use of other individuals of the same species and may result in a considerable variability of PT values. Thus, the question arises how much individuals should be radio-tracked to get robust PT values which reflect the behaviour of the whole population. To evaluate the impact of the number of tracked animals on the variability of PT values Monte Carlo simulations are conducted in order to estimate a minimum number of animals which should be tracked in order to obtain a robust PT value.

MO143

Beyond SFO? Residue decline on food in the bird & mammal pesticide risk assessment

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Residue decline data on possible food items for bird and mammals are a key factor in order to determine residue uptake to estimate exposure to plant protection products via diet by birds and mammals. Within the risk equation in the current EFSA wildlife guidance residue decline is represented by the multiple application factor (MAF) and the time weighted average factor (TWA) to calculate toxicity exposure ratios. The MAF gives estimation for increase residue uptake for more than one application and the TWA relates to a constantly decreasing residue uptake over reasonable averaging time. Both factors presented in current guidelines covering Ecotoxicology and Environmental Fate are recommended to be calculated with single first order (SFO) kinetics. In other words, a constant decrease of the substance over time. In the current EFSA guidance about risk assessment for bird and mammals details are given how residue decline studies on potential food items should be performed under field conditions. However, often the results of these studies do not follow a SFO kinetic with an acceptable fit. This can be caused by e.g. photolysis, plant growth, residue uptake after application or other compound and matrix-related reasons. Surrogate SFO kinetics as proposed in the modelling decision tree of the current EFSA kinetic guidance document underlay very conservative assumptions with emphasis on the slow phase or DT_{90} values of bi-phasic kinetics. This is because the focus of the current kinetic guideline is on the evaluation of environmental fate studies in soil or water / sediment systems deriving conservative trigger values or endpoints. In contrast, a higher tier birds and mammals risk assessment should reflect real exposure as realistic as possible. Therefore, we tested an alternative kinetic decision tree for wild life residue decline data collected under field conditions to use such data, and propose realistic residue decline data for further use in bird and mammals higher tier risk assessments if the decrease is not following single first order kinetics.

MO144

Vole field effect studies as higher tier refinement - how many voles are enough?

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The current EFSA bird and mammal guidance gives advice on how potential adverse effects of pesticides on wild vertebrates can be assessed. One option for higher tier assessments is to conduct field effect studies to monitor potential acute or long-term effects on small mammal populations using a capture-mark-recapture design. However, the guidance does not give any details on what the study set-up should look like, and studies conducted so far vary considerably. Feasibility and effort, restricted due to time and cost constraints, have certainly an impact on the quality of higher tier data from field studies for small mammal risk assessments. One important element in this typical trade-off between needs and feasibility is the number of consecutive trapping events (i.e. number of days or nights during which small mammals are trapped) within each trapping session resulting in more or less captured individuals. Combined to trapping session, such individual-based data reflect more or less accurately the respective parameters of the populations inhabiting the fields and their surroundings. Here, we examine how many consecutive trapping events are desirable per trapping session to obtain representative data of such populations. We analyse data from different field studies to evaluate the effect of additional trap nights on relevant endpoints (e.g. estimated local population size). In particular, the general variability at a trapping event and the change in the minimal detectable effect sizes are presented. As a second step, we calculate and compare estimates of survival rates, recapture probabilities and abundances using capture-recapture models provided in the MARK tool (version 9.0). Finally, we discuss the implications of the results for the design of small mammal field effect studies.

MO145

HONEY BEES DO NOT LIKE BE(E)ING CONFINED

J. Lenoir, SynTech Research

The currently required setup for evaluation of side effects of plant protection products on honey bees under semi-field conditions is to expose a bee hive to a treated crop for a specific duration in a confined environment (tunnel tents). As large as these tunnels can be, honey bees are enduring stressors challenging most of the time the reliability of the results of the studies. In addition to major stress of confinement, bees must deal with disturbances from multiple transports, visits, samplings and other assessments in a very short period of time. A honey bee colony in its hive represents a well-organized and effective group of insects, which implies an accurate regulation of conditions in the hive (temperature and moisture regulation, balance between colony needs between larval rearing, food consumption and food storing). In the experimental scenario of confinement and multiple assessments, such conditions are easily unbalanced (enhanced changes in environmental conditions, food supply, extensive hive visits, etc). The first observation of the consequence of these changes is the unusual escaping behavior: workers try to escape the tunnels instead of foraging, increasing the mortality rate of the caste dedicated to supply the hive with food. It also represents the first step in the disturbance of the test hives and their possible collapse. In view of these recurrent situations, we assessed a possible way of reducing the main stressor of confinement by testing effects of hive positioning in the tunnels. Usual side effect assessments were performed: colony conditions, mortality and flying activity assessments along with entrance activity assessment in groups of tunnels with different hive positioning. Results of the study provides additional information to improve the experimental semi-field study designs in order to allow side effect tunnel studies to achieve their intended purposes. \n Keywords: honey bees, tunnels, stress, hive position. \n

MO146

The interpretation of non-target arthropod field studies

F.M. Bakker, Bakker Consultant; S. Aldershof, Bioresearch & Evaluation

The execution of non-target arthropod (NTA) field studies generally follows a reasonably standard design as described by deJong et al (2004). In the same document quality/reliability criteria for such field studies are outlined. However, missing is formal guidance on the interpretation of field study results for regulatory purposes. Brock et al. (2015) propose a procedure for aquatic mesocosm studies that combines NOEC's and MDD's in a harmonised way and provide guidance on the reporting of these results. Their procedure to categorize taxa on basis of MDD results and the architecture of both their decision scheme to assess the adequacy of study results for regulatory purposes and their decision scheme to derive effect classes can be amended to fit NTA field studies. With this poster we focus on so-called off-field or off-crop studies. These studies, originally described as "terrestrial mesocosms" (Bakker & Miles, 2006) are also aimed at deriving hypothesis based (NOEC-type) endpoints. An important difference between aquatic and terrestrial mesocosms is the diversity and abundance of taxa and related ecological profiles. Using the same criteria as proposed by Brock et al. (2015) would not provide a balanced evaluation for terrestrial systems, due to the higher diversity in these systems. We propose to use the statistical distribution of Minimum Significant Differences (MSD's) to derive reliability criteria in a taxon-based manner. In addition, we propose to contrast individual MSD's to results obtained from reference treatments to infer whether the exposure scenario was adequate (not finding a reduction when expecting one would imply a potential issue). Together these outcomes may be integrated in a decision scheme to derive effect classifications, akin to the scheme for aquatic studies. With this poster we propose a first draft of such a scheme.

MO147

Impact of Pesticides/ Chemicals on People, Environment and Wildlife Due to Lack of Regulation, Case Study Iran

A. Mahdavi, University of Tehran/ and Sustainable agriculture and environment. / Environmental Toxicology

Pesticide/ chemicals regulations in developing countries lack a proper infrastructure, is not adequately developed and so does not completely support health of people and environment in these countries. The situation is worst in the MENA/ NENA regions due to different problems and so these areas of the planet are suffering a long decade's loss of human and wildlife loss and environmental pollution. Shocking evidences were opened last year by myself in the 2nd CMS preventing poisoning workshop including big losses of migratory birds in toxic wetlands connected to rice paddies and other source/ non source polluted water (Mahdavi, 2017, Toledo Spain for the CMS, CMS COP12 report in Manila). Iran a country very diverse in land, nature and culture continues to be a good market for imported, mostly unknown pesticides and chemicals from West in the past and in recent years from industrial Asian countries like China and India. In the past Iran was getting good information, consultations and support for all its imported products but now this support stopped. As a result, people, environment and wildlife in Iran were exposed to these toxic compounds and their metabolites during past decades. According to my long studies and also a recent field study for this paper pesticide and chemicals regulations is not in a good situation in the MENA/ NENA regions including in Iran. A high percent of wildlife loss is because of deliberate/ undeliberate poisoning and also no care about health of people exposed to these big array of toxic compounds, mixture of compounds with high possibility of synergism and recently added to it biocides. Banning of dangerous pesticides or chemicals is just a gesture with no further enforcement. Diazinon a very harmful insecticide and an undetachable compound from Iranian ecosystems is a good evidence for this. Wide use of other wildlife poisoning compounds like second generation anticoagulant rodenticides which are already banned in most countries, poison-baits, some veterinary drugs like diclofenac and lead poisoning due to lead ammunitions added to this poisoning scenario against the breaking populations of Iran wildlife.

MO148

Application of VandA to the Northern Italy

F. Galimberti, ICPS International Centre for Pesticides and Health Risk Prevention / Public Health; G. Azimonti, ICPS International Centre for Pesticides and Health Risk Prevention; A. Moretto, Università degli Studi di Milano

VandA is an innovative and easy-to-use tool to visualize the pesticide surface water contamination, assess the potential pesticide risk and address where to introduce mitigation measures to reduce the contamination and control the risk in the surface water environmental compartment (SETAC Rome 2018; VandA – Visualize and Assess: a tool for the pesticide risk mitigation in surface water). Recently, ISPRA, the Italian National Reference Institute for Environmental Research and Protection, published a National report on pesticides in water resources [1]. The scenario described in the report is drastically negative all over the Italian territory, mainly in the surface and ground water of the Po Plain in the

North of Italy. This resulting analysis highlights a critical situation which seems in contrast with the local results which shows how the latest strategies implemented by the four main Regions of the Po plain are continuously improving the agricoltural environment, enchancing the ecological state of water resources, in line with objectives of the Directive 2009/128/CE on establishing a framework for Community action to achieve the sustainable use of pesticides. Aim of the present work is to run the VandA tool on pesticide residue data in surface water of the four main Regions in the Po Plain, in order to show the trend of contamination in the last years and how the mitigations applied are improving status of surface water in agricultural areas. [1] Rapporto nazionale pesticidi nelle acque dati 2015-2016. Edizione 2018. 282/2018. ISBN: 978-88-448-0848-8

MO149

Mit-Mesw

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The authorization process for plant protection products (PPP) requires, among others, the assessment of surface water contamination deriving from the use of each product. In some cases, the application of PPP might present some critical features for the aquatic environment and the application of mitigation measures to reduce the exposure are mandatory to protect the aquatic life. In addition, on a larger scale, the adoption of mitigation measures could bring to an improvement of the quality conditions of natural resources, with valuable social and economic advantages for the agricultural sector. Surface water contamination by PPP occurs mainly from three different routes: runoff, spray drift or drainage. In Italy, due to the characteristics of the territory, runoff and spray drift are the two major routes of contamination. Different pesticide risk mitigation measures can be adopted to reduce the exposure coming from these two routes of contaminations: anti-drift nozzles, specific equipment, buffer strips (vegetated or not), hedgerows etc. The combination of different mitigation measurements can produce a high level of mitigation. These huge amount of combinations cannot be included into a label; therefore, a simplified solution was adopted in Italy. In the label, the mitigation coming from modelling, performed according EU criteria is indicated together with the percentage of reduction of dose necessary to obtain a safe use. On the other hand, a harmonized value of reduction has been assigned to each possible mitigation measure. These values are in line with those proposed by the MAgPIE project (Mitigating the Risk of Plant Protection Products in the Environment), with other research projects (i.e. STRIPE-Ireland) and with some national researches and expert judgement. The possible mitigation measures applicable in Italy have been listed in a guidance document and an easy-to-use tool has been developed. The tool is called Mit-Me$_{sw}$ (MITigation MEasures for Surface-Water). This tool, through a series of combo-menus and images, aims at helping farmers in choosing the best equipment and agronomic practices to mitigate the pesticide risk, and pesticide risk assessors in quantifying the percentage of risk reduction due to each singular/combined adopted mitigation measures.

MO150

WaterProtect - a joint European initiative to protect drinking in water rural and urban environments: the experience of the lower Llobregat River basin (NE Spain)

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Water quality in Europe is under constant pressure and this represents a big threat for our drinking water resources. In this context, the EU project WaterProtect aims at developing new solutions and tools to effectively uptake and identify management practices and mitigation measures to protect drinking water resources threatened by agricultural, urban and industrial pressures. This will be conducted in seven action labs spread in the European territory that represent different realities and problems in terms of water governance. From the experiences in the different action labs, lessons learnt will be upscaled so that they can be applied to improve water governance in any other location. The lower basin of the Llobregat River (NE Spain) is one of the WaterProtect action labs. This area is located in a densely populated area, as it is the metropolitan area of the city of Barcelona. Because of this, groundwater is intensively exploited for different uses (production of drinking water, agriculture, and industrial activities), and the river water serves as a source for drinking water production. Overall, water quality in the area is highly impacted by urban and industrial activities and to a less extent by agriculture, also present in the area. In the framework of WaterProtect, a multi-actor participatory approach was used to identify water management needs in the lower Llobregat River basin and to engage different water actors in water governance. A participatory monitoring approach was used to design a monitoring campaign to cover knowledge gaps regarding pesticides and nitrates in the area. A practical collaborative open access tool that integrates water quality data has been created and will be internet freely available to the general public. Finally, in order to improve water quality in the area and reduce the impact of agricultural activities, selected best management practices are being promoted among farmers and two techniques for biorremediation of water polluted with pesticides are being tested. *Acknowledgments:* This work has received funding from the Government of Catalonia (2017 SGR 01404), the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the project BECAS (CTM2016-75587-C2-2-R), and the EU Horizon 2020 Programme (No. 727450). This presentation only reflects the authors' views and the Commission is not responsible for any use that may be made of the information it contains.

MO151

Modelling real VFS experiments with a new VFSMOD version - calibration and uncertainty analysis with DREAM-ZS

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The most widely implemented mitigation measure to reduce transfer of pesticides and other pollutants to surface water bodies via surface runoff are vegetative filter strips (VFS). To reliably model the reduction of surface runoff, eroded sediment and pesticide inputs into surface water by VFS in a risk assessment context, an event-based model is needed. The most commonly used dynamic, event-based model for this purpose is VFSMOD. While VFSMOD simulates reduction of total inflow (?Q) and reduction of incoming eroded sediment load (?E) mechanistically, the reduction of pesticide load by the VFS (?P) has until recently been calculated exclusively with the empirical Sabbagh equation. The latest version of VFSMOD (v.4.4.0) includes further pesticide trapping options, notably the Sabbagh equation with user-defined regression coefficients and a regression-free mass-balance approach. The new VFSMOD version was systematically tested against real experimental data. Four studies with 16 hydrological events and 31 combinations of hydrological event and compound were selected from the experimental data compiled by Reichenberger et al. (2019), representing different levels of data availability and uncertainty. A first set of simulations was conducted with parameterization according to SWAN-VFSMOD and the three pesticide trapping options mentioned above. The Sabbagh equation with optimized coefficients from Reichenberger et al. (2019) and the original Sabbagh equation overestimated pesticide trapping efficiency ?P for the low range of measured ?P, while the mass balance approach yielded the most conservative results. These observations were due to an overestimation of ?E with the SWAN-VFSMOD parameterization. The simulation results suggest that the SWAN-VFSMOD parameterization of saturated hydraulic conductivity is too conservative, while the parameterization of sediment filtration is too optimistic. In a second step, a maximum-likelihood-based calibration and uncertainty analysis was performed for each hydrological event and the target variables ?Q and ?E with the DREAM-ZS algorithm. One aim of the DREAM analysis was to help improve the parameterization methodologies for the infiltration and sediment filtration modules for regulatory VFS scenarios. Subsequently, the three pesticide trapping equations were applied in predictive mode to elucidate which equation performs better in which situation (e.g. soil type, Kd, characteristics of runoff/erosion event).

MO152

The future of risk assessment is not risk assessment: could improved farmland biodiversity mitigate marginal risk assessments?

A. Lawrence, Cambridge Environmental Assessments / Institute of Environmental Toxicology

Recent experience suggests that evaluations of environmental risk assessments for plant protection products (PPP) by Member States/EFSA are becoming more conservative. This may be attributable to issues such as exposure to multiple stressors at the field or catchment level. There is also concern that farmland biodiversity is impoverished and that even minor, transient, toxic insults cannot be tolerated. Media reports on biodiversity loss and negative effects of PPP may add to this. Notifiers are investing more and more resources in higher tier risk assessments, such as multiple field studies. Increasingly, however, any uncertainty associated with the risk assessment or studies is sufficient to conclude no acceptable risk or no safe use, despite a considerable weight of evidence to the contrary. With the loss of neonicotinoids we may turn to older chemistry, which is also under threat from these issues. There is a need, therefore, to explore ways in which such concerns may be overcome, to keep key crop protection tools on the market while also ensuring a high level of environmental safety. There is strong evidence from various demonstration farms and industry initiatives that intensive agriculture and PPP use can go hand in hand with vastly improved biodiversity –

examples will be provided. This can be achieved through crop selection, in-field measures and intelligent margin management. This therefore begs the following questions: If resources were invested in measures to demonstrably improve farmland biodiversity, could concerns regarding the impact of transient, minor effects of PPP be alleviated? Could a more resilient agroecosystem be considered better able to buffer minor effects of PPPs, which increasingly result in recommendations of non-approval for key crop protection tools? The means to achieve this already exist and were summarised in a recent report for the MagPIE project (Hackett & Lawrence, 2014). An important option with significant potential is multi-functional field margins (MFFM). MFFM can mitigate exposure of off-field habitats through the attenuation of spray drift and/or run-off, while also being managed to promote biodivesity. Label requirements for management of no-spray buffers and filter strips offer a mechanism to ensure compliance. Other available tools offer in-field benefits. This poster aims to further discussion around how we may maintain access to key PPPs by promoting farmland biodiversity using existing management options.

MO153
Changes foreseen according to EMAs new draft guideline on the environmental risk assessment (ERA) of medicinal products for human use
R. Wess, Innovative Environmental Services (IES) Ltd. / Regulatory Consulting
The Committee for Medicinal Products for Human Use (CHMP) has issued the ERA new draft guideline (EMEA/CHMP/SWP/4447/00 Rev. 1) on 15 November 2018. As the commenting is possible until the 30 June 2019 (deadline for comments) the poster intends to summarize some of the most important changes in order to facilitate the timely submission of comments. Some issues, being in contradiction to the recent questions and answers document of 2016 (EMA/CHMP/SWP/44609/2010 Rev. 1) are that generally all HPLC / OPPTS methods are phased out.\nMany amendments represent the scientific or regulatory progress:\n- NOEC phased out except for limit studies (thus always EC10 calculation required)\n- Earthworm reproduction study instead of the acute test\n- Study rating and summaries required\n- Reference/Harmonisation to/with Water Framework Directive (WFD) Guidance for deriving EQS (Guidance Document No.27, Technical Report-2011–055)\n- Formal submission item characterisation\n- Full dataset for pH-dependency on lipophilicity (Dow); i.e. determination Dissociation constant and Kow (of the neutral molecule)\n- Requirement to undisclose the relation of the assessor to the applicant\n- Specific study demands for potential endocrine disruptors and antibiotics\n- Use of DT50 values for the EU annual average temperature of 12 °C\n- Sediment organism effect testing switched to phase II, Tier A & increased assessment factor of 100 for Chironomid life cycle EC10\nFurthermore some changes signify a change in the structure of the assessment approach:\n- PEC correction based on marketing data is phased out\n- Thus it is much more important to have data allowing the derivation of the fraction of substance excreted (F excreta) - this has impact on potentially already ongoing pharmacokinetic studies\nSome dubiety remain due to the absence of any hint on an acceptable approach for the calculation of an activation energy for use in the Arrhenius equation (correction of DT50 to 12 °C). Also no standard for the study summaries is given however application of the OECD templates is nearby. Hyalella is not mentioned in the guidance any more, but due to the reference to WFD may be regarded anyhow.

MO154
Incorporating bioavailability in aquatic risk assessment of pharmaceuticals
K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology; L. Vandeneynde, Arche consulting; F. Verdonck, ARCHE; P. Van Sprang, Arche consulting
About 75% of human pharmaceuticals are ionisable, with the ratio between neutral and charged forms of API being determined by both the environment (pH) and the acid-base behavior (pKa) of the active pharmaceutical ingredient (AP)I. Theoretical considerations and some recently published empirical work indicate that the unionized form of an ionizable API is usually more easily taken up, more bioavailable and more toxic than the ionized form. However, although the effect of pH on API availability can amount to one order of magnitude per pH unit change, we are unaware of any regulatory risk assessment framework that takes this into account. For instance, neither the new draft guidance document on human pharmaceutical risk assessment nor the derivation of environmental quality standard (EQS) under the water framework directive (WFD) considers bioavailability. This is much in contrast with what is now common practice in metals risk assessment, where effects on ecotoxicity of factors such as pH, DOC and waters hardness are accounted for using semi-mechanistic biotic ligand models (BLM). Here, focusing on pH as a factor, we use a case study with two pharmaceuticals, the anti-inflammatory drug diclofenac (acid, pKa=4.0, more ionized at increasing pH) and the beta-blocker propranolol (base, pKa=9.4, more ionized at low pH), to show how the predicted no effect concentration (PNEC) or EQS can vary across Europe (based on the FOREGS database), if bioavailability, through ionization, is take into account. The EQS for diclofenac is currently at 100 ng/L (based on histological effects on rainbow trout in a test at pH 7.4), but would range between 10 to 794 ng/L (10-90th percentile) if only the unionized form is assumed bioavailable, with many low values occurring in Northern Europe (mostly low pH). Similarly, propranolol, with a reported PNEC of 200 ng/L (based on a sea urchin test at test pH of 8.1), shows varying PNEC values from 130 to 9555 ng/L (with most low values occurring in Southern Europe, high pH). Our analysis shows that not taking into account bioavailability can result in both under or overestimation of true risks, depending on pH of the ecotoxicity tests, acid-base behavior of the API, and geographic location (or local pH). Taking into account bioavailability would improve this problem and could help better prioritization of API in terms of risk reduction.

MO155
SETAC Dung Organism Toxicity Testing Interest Group
J. Roembke, ECT Oekotoxikologie GmbH
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MO156
SETAC Pharmaceuticals Interest Group
G. Maack, German Environment Agency / Ecotoxicological Assessment
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Effect Modelling for Regulatory Risk Assessment: Current Applications and Future Directions (P)

MO157
Use of algae modelling as a tier 2c refinement within the framework of the EFSA TKTD opinion
J. Witt, Bayer AG / Environmental Safety; J. Hager, Bayer Ag; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology; T. Preuss, Bayer Ag / Environmental Safety
Algae growth inhibition tests measure growth inhibition for constant exposure to a chemical. Real-world exposure is often much more variable. The use of a mathematical algae model offers the opportunity to predict the effects of time variable exposure in silico, and therefore improves the realism of risk assessment. While effect modelling for risk assessment was performed and evaluated on a case by case basis in the past, the recently published EFSA TKTD opinion has made an important step towards a standardised effect modelling framework in risk assessment. Evaluation of the algae model of Weber et al. within the TKTD opinion yielded a two-sided result: the predictive power of the model was confirmed under constant and optimal conditions. However, the flow-through test design used for validation was identified as the largest drawback for implementing the model in risk assessment, because of its non-standard design that has not been ring tested. Consequently, an update of the current practice of using the algae model in combination with experimental data will be required to increase regulatory acceptance. We show how the algae modelling workflow may be adapted to fit into the framework of the TKTD opinion. This includes a proposal for an alternative test design for validation experiments. We then show how the model can be used as a tier 2c refinement in risk assessment.

MO158
Proposal how to use measured exposure concentrations in calibration and validation experiments as input for TKTD models
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With the increased use of TKTD models in risk assessment, there is an increasing need for standardised procedures. Many recommendations have recently been given in the EFSA TKTD opinion. One point, however, still remains open: how should the measured exposure concentrations be used as an input for TKTD models? This may seem straightforward at first sight. However, exposure concentrations may vary over time in the experiments, and there are different ways to address the variability, e.g. arithmetic mean, geometric mean, a fitted decline curve, or linear interpolation. Different approaches may be more or less appropriate in different situations. The chosen approach may have a huge effect on the temporal exposure pattern and may therefore have a considerable influence on the final outcome of the modelling. We propose a standardised default procedure how to use measured exposure concentrations in calibration and validation experiments as an input for TKTD models, respecting existing guidance (AGD corrigendum). These procedures are being used within Bayer for several years and proved to be applicable to a broad data set from different species (algae, macrophytes, aquatic invertebrates and fish). The recommended procedures depend on experimental setup (flow-through, semi-static, static) stability of the test item quality and availability of data Well-reasoned exceptions may be made on a case-by-case basis.

MO159
Scientific opinion about TKTD models to assess risks of time-variable exposure for pesticides in edge-of-field surface waters
A. Focks, Wageningen Environmental Research / Environmental Risk Assessment Team; M. Arena, EFSA - European Food Safety Authority / Pesticides; T. Brock, Wageningen Environmental Research / Environmental Risk Assessment Team; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences; S.

Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; S. Duquesne, German Environment Agency; A. Ippolito, EFSA - European Food Safety Authority / Pesticides Unit; M. Klein, Fraunhofer IME / Ecological chemistry; M. Reed, HSE; I. Teodorovic, University of Novi Sad / Department of Biology and Ecology, Laboratory of Ecotoxicology (LECOTOX)
In 2018, the Panel on Plant Protection Products and their Residues (PPR) of the European Food Safety Authority (EFSA) published a scientific opinion (SO) on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) models in regulatory risk assessment of pesticides for aquatic organisms (*EFSA PPR, 2018. EFSA Journal 2018;16(8):5377*). This SO gives an overview and evaluates the state of science of existing TKTD models for lethal and sublethal effects on animals and primary producers. It is concluded that GUTS models (General Unified Threshold models of Survival), which account for lethal effects on animals, are well established and can be used in the risk assessment scheme to assess the risk of time-variable exposure. DEBtox models (Dynamic Energy Budget toxicity models) account for sublethal effects of pesticides on growth and reproduction. They are considered to be in an advanced state but not yet ready to be used for regulatory risk assessment; the SO provides some suggestions for improving them and making them more suitable for this purpose. Models accounting for the effects of pesticides on primary producers have been also evaluated. Currently, species–specific models have been developed for *Lemna* sp., *Myriophyllum* sp, and two algae species. A conclusion about the state of science for these species-specific models suggests that the *Lemna* model is suitable for use in regulatory risk assessment, given that some remaining aspects of model applications such as better documentation of optimisation methods and sensitivity analyses are improved, while some shortcomings prevented to recommend the *Myriophyllum* and algae models as fit for purpose. The poster gives an overview about the principles of these different TKTD models and summarises the conclusions presented in the SO.

MO160
Beyond GUTS: DEBtox and primary producer TKTD models in ERA of pesticides
M. Reed, HSE; M. Arena, EFSA - European Food Safety Authority / Pesticides; T. Brock, Wageningen Environmental Research / Environmental Risk Assessment Team; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; S. Duquesne, German Environment Agency; A. Focks, Wageningen Environmental Research / Environmental Risk Assessment Team; A. Ippolito, EFSA - European Food Safety Authority / Pesticides Unit; M. Klein, Fraunhofer IME / Ecological chemistry; I. Teodorovic, University of Novi Sad / Department of Biology and Ecology, Laboratory of Ecotoxicology (LECOTOX)
In 2018, the Panel on Plant Protection Products and their Residues (PPR) of the European Food Safety Authority (EFSA) published a scientific opinion on the state of the art of Toxicokinetic Toxicodynamic (TKTD) models in regulatory risk assessment of pesticides for aquatic organisms (*EFSA PPR, 2018. EFSA Journal 2018;16(8):5377*). In this scientific opinion it is concluded that Dynamic Energy Budget theory for ecotoxicology (DEBtox models) and models for some primary producers are not yet sufficiently developed to use them in the risk assessment scheme when assessing the risks of time-variable exposure. This poster considers how these models could be used as a Tier-2 option for environmental risk assessment (ERA) and what still needs to be done to permit routine use. The DEBtox modelling framework, based on the DEB theory, is developed to address individual level lethal and sublethal chronic effects. It may be an appropriate approach to use in the refined chronic risk assessment scheme for aquatic invertebrates, fish and aquatic stages of amphibians, particularly when sublethal endpoints are most critical in chronic toxicity tests. Both the physiological DEB part of DEBtox models and their TKTD part need to be fully evaluated to use the model. Three primary producer models are considered, *Lemna,* algae and *Myriophyllum*. The *Lemna* model appears suitable for use in risk assessment to evaluate effects of time-variable exposure on *Lemna* growth; however, some remaining aspects of the model require further work to make applications acceptable for use in regulatory risk assessment. The algae model is also described but currently its largest drawback is that the flow-through experimental setup, used for model validation to simulate long-term variable exposures of pesticides to fast growing populations of algae, has not yet been standardised. The *Myriophyllum* model looks promising, but currently there is insufficient information available to recommend its use for ERA. For all the models presented, the poster aims to provide recommendations for future developments that will help the models be accepted for regulatory risk assessment of pesticides.

MO161
User-friendly software for survival modelling with GUTS
T. Jager, DEBtox Research / Dept of Theoretical Biology; M. Wang, WSC Scientific GmbH / Dept Efate Modelling; R. Ashauer, University of York / Environment
Mechanistic effect models are rapidly gaining interest in the context of environmental risk assessment. In 2018, this interest culminated in a dedicated EFSA opinion on toxicokinetic-toxicodynamic (TKTD) models for use in aquatic risk assessment of pesticides. This opinion focussed on three modelling frameworks; for the endpoint survival, the focus was on GUTS (the General Unified Threshold model for Survival). Since the EFSA opinion judged GUTS to be "ready to be used in risk assessment", there is now a pressing need for robust and user-friendly software to perform analyses with this model, following the proposed workflow in the opinion. In a Cefic-LRI funded project, we are currently developing such a software. The resulting software will be able to perform the procedures for calibration, testing and model predictions as outlined in the EFSA opinion. Furthermore, it will perform these analyses with minimal demands on user experience in modelling and statistics. However, this software will not be restricted to risk assessment of pesticides. It can also be used for other tasks, such as the derivation of classical LC50 values (as function of time) from standard and non-standard toxicity data, using all of the observation on survival over time. Specifically, the software will enable meaningful calibration to toxicity data that result from tests with time-varying exposure, which cannot be achieved using traditional dose-response curves. The software is planned to be released by the end of 2019, but in this contribution, we provide a sneak preview of the software's layout, algorithm, functionality and look.

MO162
On the best practice for DEB(tox) parametrisation for regulatory risk assessment
B. Goussen, IBACON GmbH / Ecological Modelling; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; A. Gergs, Bayer AG - Crop Science Division / Department of Environmental, Social and Spatial Change; T. Preuss, Bayer Ag / Environmental Safety
The development of a consistent and relevant modelling approach for the regulatory environmental risk assessment of sub-lethal effects of chemical compounds is a major challenge that starts to be addressed. Following the EFSA Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models (*EFSA PPR, 2018. EFSA Journal;16(8):5377*), models based on the Dynamic Energy Budget theory adapted for ecotoxicology are valuable tools for environmental risk assessment. In principle, such models should be fully calibrated and validated, considering both physiological and toxicological parameters all together. Nevertheless, to keep them simple and manageable, a first step towards their full acceptance by stake-holders would be to have the physiological parameters of DEB-based models independently approved by a competent expert team, and, in a second step, to only calibrate toxicological parameters from toxicity test data. Despite its simplicity, this approach bears a number of statistical flaws and might not allow for sound forward predictions. Therefore, we propose to test to what extent separating calibration of physiological and toxicological parameters may impact predictions of sub-lethal effects due to chemical compounds. To do so, we need to define appropriate calibration framework for the standard DEB model coupled with stress functions. As a first step, we identified three possible frameworks (i) The strict separation view, i.e., fix the physiological parameters to their mean regulatory accepted values and get estimates of toxicological parameters only from experimental toxicity test data then use fixed mean values of physiological parameters and estimated toxicological parameters including their uncertainty for predictions; (ii) the statistical view, i.e., use the physiological parameters as input priors to perform full Bayesian inference and get estimates of both the physiological and toxicological parameters, then use them associated to their uncertainty for predictions; or (iii) a compromise, i.e., use the physiological parameters as input priors to perform full Bayesian inference and get estimates of both the physiological and toxicological parameters, but use the fixed mean regulatory accepted values of the physiological parameters together with the estimated toxicological parameters including their uncertainty for predictions. The analysis of each of these approaches is here presented and discussed.

MO163
Dynamic energy budget modelling of Daphnia magna population dynamics - validation examples
A. Gergs, Bayer AG - Crop Science Division / Department of Environmental, Social and Spatial Change; K. Rakel, Research Institute gaiac / gaiac- Research Institute for Ecosystem Analysis und Assessment
In the context of the prospective environmental risk assessment for pesticides, individual-based population models (IBMs) allow quantitative predictions on how populations perform under chemical stress based on individual level toxicity test results. When integrated with toxicokinetic-toxicodynamic (TK-TD) models, the IBMs can be used e.g. for the evaluation of potential population level risks that may arise from time-variable exposures on aquatic organisms such as *Daphnia magna*. As regards TK-TD models, dynamic energy budget (DEB) models are particularly suitable to deal with effects of chemicals on life-history traits related to growth and reproduction, while the general unified threshold model of survival (GUTS) accounts for lethal effects. In this regard, we will showcase validation examples from a DEB based IBM, parametrized for *Daphnia magna*. For validation, we confront model predictions with population level data from various control conditions as well as for exposure situations resulting in sublethal or lethal effects.

MO164

Dynamic energy budget modelling of macroinvertebrate population dynamics - validation examples from different species

A. Gergs, Bayer AG - Crop Science Division / Department of Environmental, Social and Spatial Change; D. Liesy, Research Institute gaiac; H. Selck, Roskilde University / Dept Science and Environment; A. Palmqvist, Roskilde University / Department of Science and Environment

In the ecological risk assessment of chemicals, mechanistic effect models provide the possibility to extrapolate effects to untested scenarios. In particular, individual-based population models (IBMs) are able to make quantitative predictions on how populations perform under chemical stress based on individual-level toxicity testing. In the past, IBMs have usually been designed for specific species and have, thus, been perceived as being too complex to understand mainly due to the variety of existing approaches. The developments of more standardized model designs will facilitate their acceptability. Dynamic energy budget (DEB) models are suitable to describe life-history traits related to growth and reproduction and, when integrated in an IBM framework, allow simulating population dynamics. DEB models have been developed for a large variety of species and parameters, code and underlying data are available from the so called add-my-pet collection. We here show, for the example of different aquatic macroinvertebrates, that the same basic DEB -IBM, only changing parameter values and initial conditions (to match the experimental setup used for comparison of data and model output), is able to adequately predict population dynamics.

MO165

Dynamic energy budget modelling of Daphnia magna growth and reproduction - a validation example

A. Gergs, Bayer AG - Crop Science Division / Department of Environmental, Social and Spatial Change; K. Rakel, Research Institute gaiac / gaiac- Research Institute for Ecosystem Analysis und Assessment; T. Riebschlaeger, Bayer Ag; K. Kuhl, Bayer AG / Crop Science Division; V. Herno, Bayer SAS; T. Preuss, Bayer Ag / Environmental Safety

In the context of the prospective environmental risk assessment for pesticides, toxicokinetic-toxicodynamic (TK-TD) models are useful e.g. for the evaluation of potential risks that may arise from time-variable exposures on aquatic organisms. Dynamic energy budget (DEB) models are particularly suitable to deal with effects of toxic chemicals on life-history traits related to growth and reproduction. DEB models for chemical effects consist of two modules, a ‘physiological’ module that describes the acquisition and use of energy for growth and reproduction, and a TK-TD module that accounts for chemical stress. As regards the physiological module, the add-my-pet collection hosts DEB models, parameter estimates and underlying data for a broad range of species, including *Daphnia magna*. When combined with the physiological DEB model as derived from the add-my-pet collection, the TK-TD module can be used to analyze data from *Daphnia magna* reproduction test. A species and compound specific model validation is key to reliable applications within the prospective environmental risk assessment. In this regard, we will showcase a validation example for both the physiological DEB model as well as TK-TD module using Trifloxystrobin as an example.

MO166

Mixture toxicity with an individual-based model on the dynamic energy budget theory: effects of Cu and Zn on Daphnia magna populations

K. Vlaeminck, Arche consulting / GhEnToxLab; K. Viaene, ARCHE; P. Van Sprang, Arche consulting; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology

The current regulatory framework of risk assessment considers the effects of single substances that are assessed through standardized laboratory tests, e.g. the 21-day *Daphnia magna* reproduction test. However, in the environment, individual organisms are being exposed to mixtures of chemicals. Protection goals in EU legislation (e.g. Water Framework Directive) state that protection of populations is needed to maintain ecosystem functioning. In our research, we developed an Individual-Based Model of the Dynamic Energy Budget theory (DEB-IBM) to predict effects of metal mixtures on *Daphnia magna* populations. The DEBtox model was calibrated using apical effects (i.e. growth, reproduction and survival) of Cu and Zn assessed in standardised tests. To validate our model and determine the most appropriate mixture toxicity mode at the population level, a population experiment (75 days) with *D. magna* exposed to Cu and Zn (both single and mixtures) was performed. We tested three implementations of mixture toxicity in DEB-IBM: independent action (where effects of the compounds are considered independent), concentration addition (where the compounds have the same toxic mode and act as dilutions of each other) and effect addition (where the compounds have the same toxic mode but effect levels are summed). Each implementation was evaluated for predictability against the data of the population experiment using the normalised mean square error (NMSE). All three implementations perform well compared to the data, indicating that none of the mixture models is preferred at the population level. We extrapolated the experiment with DEB-IBM to untested concentrations and determined effect levels for Cu and Zn based on equilibrium population density. The effect levels of the three mixture toxicity implementations were compared to assess the applicability of our model for mixture toxicity risk assessment at the population level. The current implementation of mixture toxicity in DEB-IBM could accurately predict mixture toxicity effects observed in a population experiment. The concentration addition model seemed to be the more conservative mixture toxicity mode. More fine-tuning and comparison with experiments is needed to further validate our approach and increase confidence in the predictions.

MO167

Implementing realistic biological variability in an individual-based Dynamic Energy Budget model

J. Koch, GhEnToxLab (Ghent University) / Applied Ecology and Environmental Biology; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology

One of the biggest advantages of individual-based population models (IBMs) is the possibility to simulate biological variation among individual animals. Inter-individual variation is known to promote the ecological success of populations by making them more resilient to environmental changes and stress events. While inter-individual variation can be measured in virtually all populations, reproducing such variation in population models accurately is not always straightforward. That is mainly because variation can be measured in apical endpoints like development time, size at a certain age or life stage, or reproduction success, but not on the underlying physiological parameters of the organism. In IBMs that make use of the Dynamic Energy Budget (DEB) theory, the development of an organism depends on 12 primary parameters, all of which potentially vary among individuals to some extent. While previous studies included stochastic scatter for individual parameters (one at a time), the degree of variation in this parameter has always been chosen rather arbitrarily. In this study we used experimental data on the development time and brood sizes of the copepod *Nitocra spinipes* reared at control conditions to make realistic estimates of the variability in DEB parameters for this species. As a first step, a global sensitivity analysis was performed to identify the parameters that are linked most closely to the observed endpoints. Subsequently, stochastic scatter was introduced to these parameters by drawing them from a probability distribution (multiple distribution types were tested and compared ranging from uniform to log-normal distributions). The degree of variation per parameter was adjusted by means of an optimisation algorithm that makes use Monte Carlo simulations. The Kolmogorov-Smirnov test statistic was used to assess the difference between measured versus simulated data distributions. A simulated annealing approach was used to minimise the difference and hence optimise the variation parameters.

MO168

Toxicokinetic and Toxicodynamic modelling of the fungicide tebuconazole in the benthic organism Chironomus riparius

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Sediment is a sink for thousands of man-made chemicals that may impact structure and functioning of this key compartment, raising need to evaluate associated risk for aquatic organisms. In this context, one major issue is to better characterize the uptake and biotransformation of the contaminants in the organisms in order to identify the internal dose that may trigger a biological response. For this purpose, Toxicokinetic-Toxicodynamic models are increasingly used. In the present study we investigated for the fate of the antifungal tebuconazole in the benthic organism *Chironomus riparius* that is used as a model in regulatory hazard assessment. To this end, chironomids were first exposed to artificial sediment spiked at 1000 µg/g d.w. in order to identify its biotransformation product(s) using HRMS/MS. Then, TK experiments were implemented consisting in exposure to tebuconazole at 200 µg/g d.w. during 24h of uptake followed by 96h of depuration. An one compartment model was used to describe the time course of the tebuconazole and the major identified metabolite. This revealed particular fast uptake (i.e. steady state reached in one hour) and very fast depuration of the chemicals. Such TK could be related to the tebuconazole bound to the ingested sediment, still present in the organisms during the analysis due to the absence of a gut purging phase. Nevertheless, the identification of an oxidative product of the tebuconazole, demonstrated that biotransformation is also involved in the fate of this fungicide in chironomids and therefore bioavailable. Then, we implemented TD experiments by exposing chironomids to a concentration range of tebuconazole (50-100 mg/g) during 14 days and growth and survival were assessed along all experiment. From these data, we implemented a General Unified Threshold model for Survival (GUTS) based on both stochastic and independent death hypotheses that highlighted decreasing LC50 values along the exposure time. The results also showed reduced growth with increasing exposure time and concentration. Although these data were integrated in a DebTox model, the growth curves did not fit so well with actual values, likely because of the use of the Von-Bertalafny growth model that is not suitable to describe the growth of insects. Nevertheless, effect on the growth raised the question of the involved mechanism(s) in non-target species and its conservation along evolution since such effect of the tebuconazole is also reported in fish.

MO169
Screening for protonophoric uncouplers
A. Ebert, Helmholtz Centre for Environmental Research UFZ; K. Goss, Helmholtz centre for environmental research - UFZ / Analytical Environmental Chemistry
Test procedures and their evaluation in current regulatory risk assessment are mainly focused on neutral (uncharged) compounds. Yet, a substantial part of the compounds to be assessed is ionisable. For these, pH-dependence of toxicity is an important factor. One such pH-dependent effect is protonophoric uncoupling, where a weak acid shuttles protons H^+ across the inner membrane of the mitochondria, and thereby decreases/dissipates the proton gradient created by proton pumps of the respiratory chain. This gradient is essential for ATP-synthesis, and its dissipation leads to a toxic effect. Due to recent advances in the prediction of anionic permeation through lipid bilayers, we were able to develop a purely mechanistic biophysical prediction model of pH-dependent uncoupling toxicity. Our model describes a steady state situation, where the net flux of the protonophore across the membrane is zero. At the same time, we assume a toxic effect to occur once the flux of shuttled H^+ reaches the same order of magnitude as the opposing flux of H^+ created by the proton pumps. Important input factors are physiological data, like the pH- and potential-gradient across the mitochondrial membrane, as well as the fractionation (pKa) and the permeability of the weak acid. The core input is the anionic permeability of the uncoupler. At this point, our mechanistic model is well suited to predict minimal toxicities of ionisable compounds, as well as pH-dependencies and their direct cause (like ion trapping or limitations by the neutral permeability). But to avoid underestimation of toxicity due to uncoupling, heterodimer permeation will have to be included in the model. The advantage of our model over already existing quantitative structure-activity relationships (QSARs) is the ability to account for pH- or potential-gradients across the membrane. Also, the mechanistic nature of the model allows to draw direct conclusions on the factors that limit the uncoupling activity. Furthermore, because the mechanistic model should not be limited to certain classes of chemicals, it can be used as a screening tool to check large databases for possible uncouplers, and predict their pH-dependent uncoupling toxicity. This may facilitate the right choice of relevant pH ranges of toxicity assays.

MO170
Modelling vs. experimental testing of chemical toxicity of environmental mixtures of pesticides on freshwater algae
K.A. Sandblom, T. Backhaus, University of Gothenburg Sweden / Department of Environmental and Biologigal Sciences; N. Corcoll, University of Gothenburg, Sweden / Biological and Environmental Sciences
Mixture toxicity modelling has emerged as a promising tool for mixture toxicity risk assessment, however, such models have not been fully validated against experimental testing. In our project we want to take a step further and compare risk assessment from mixtures of pesticides on algae between modeling and experimental testing using real environmental mixtures, and compare risk assessment models based on a single species-oriented approach vs. a species sensitivity distribution (SSD) approach. In this study, a field sampling was carried out in three agricultural streams, presenting different levels of pesticide pollution (SE Sweden), in September and October 2016. For each sampling site, pesticide levels in surface water and from passive samplers (e.g Chemcatchers) were analyzed. The risk of identified pesticide mixtures on freshwater algae was assessed by mixture toxicity modelling and with ongoing laboratory ecotoxicity tests using the field passive sampler extracts. Our ecotoxicity tests are performed in an optimized semi high-throughput assay (24/96-well microplates) for the freshwater algae *Raphidocellis subcapitata* based on algal growth, following the OECD protocol (201), to increase the capacity of ecotoxicological testing. Based on mixture toxicity modeling, five pesticides (three herbicides and two fungicides) with different modes of actin (MoA) were identified as the toxicity drivers in studied rivers. The toxicity of these compounds on algae is tested in two settings: single compounds exposure *vs.*artificial mixture. This will allow us to validate the precision and utility of the current prediction models - concentration addition (CA) and independent action (IA) - that are based on a single-species approach. The same workflow will be used to generate ecotoxicity data for five different species of freshwater algae from distant phylogenetic groups (i.e. Chlorophyta, Rhodophyta, Bacillariophyta, Cyanophyta and Chrysophyta), in order to produce species sensitivity distribution curves (SSD) and infer a community approach. The SSD approach is of particular interest since different species are expected to present different sensitivities to toxicity drivers (with different MoA) when exposed alone and in combination. Mixture toxicity modelling combined with the SSD approach might give us new insights on how to expand the mixture toxicity risk assessment for algae on a community level and use this tool for a better protection of the aquatic environment.

MO171
A recipe for the development of High-Accuracy QSAR models based on toxic mechanisms of action
P. Bicherel, F. Sahigara, KREATiS; P. Thomas, CEHTRA SAS / Ecotoxicology and Risk Assessment; F. Bauer, KREATiS
Often, the ultimate objective of the QSAR model is to prioritise chemicals for further testing rather than to predict an exact experimental value. Therefore most of the QSAR models are developped for screening pruposes and tend to lack the precision required to replace experimental studies. The OECD settled five criteria of validity to ensure the reliability of a model, and its prediction consequently. However these conditions remain general, plus they are not always and completely satisfied in practice. For this reason, it seems necessary to distinguish between QSAR models created to provide approximations of experimental values and those specially designed to substitute for specific experimental endpoints, the High-Accuracy QSAR models (HA-QSARs). In order to develop this kind of models, the five validation criteria of the OECD need to be elucidated and specified. Furthermore, the mecanistic explanation suggested as facultative appears to be critical, especially in the field of the toxicology. Recommandations are given here to base the models on toxic Mechanism of Action (MechoA) instead of the chemical structure. The aquatic toxicity predicted with commonly used QSARs and HA-QSAR is compared to experimental values which have been validated to be reliable. The analysis shows that the HA-QSAR gets closer result than others to those reference values. Enough confidence can be granted to HA-QSARs to such an extent that they should be considered as serious candidate to remplace experimental test when it is not necessary. [PB1]Abstract. Max 2500 characters, including spaces.

MO172
Why the margin of safety in first tier risk assessment is meaningless - A comparison of first tier with results from realistic TDTK and population modelling
J. Kleinmann, WSC Scientific GmbH; M. Wang, WSC Scientific GmbH / Dept Efate Modelling
In first tier risk assessment of birds and mammals, the risk posed by pesticides to species is indicated by the LD_{50} of a toxic substance and the daily dietary dose of the corresponding species. A safety factor of ten is applied to cover any uncertainty (e.g. regarding inter-species variability and the uncertainty about the slope of the dose response curve). While one might interpret this safety factor as a margin of safety, in reality the margin of safety can be very different from this safety factor. Many substance specific properties, which may strongly influence the risk (e.g. dose-response curve, uptake and elimination rates), are completely ignored but may be critical for the risk. In the first tier the risk is considered to be low, if the DDD of the assessed species is ten times lower than the LD_{50} of the substance. However, since the LD50 is not a “safe dose”, such as e.g. the NOAEL, it is not possible to identify the margin of safety in this calculation. To quantify the margin of safety of the first-tier risk assessment, an existing population modelling framework (POLARIS) has been combined with a TKTD model (GUTS) to simulate the risk from various toxic substances on populations of wild mammals. It is shown, that the margin of safety of a first tier risk assessment strongly depends on substance specific parameters that are not considered in the first tier.

MO173
Modelling life history traits of amphibia for population modelling
J. Kleinmann, T. Braasch, WSC Scientific GmbH; M. Wang, WSC Scientific GmbH / Dept Efate Modelling
In a recent scientific opinion, EFSA (2017) proposed the inclusion of amphibian and reptile species in the process of pesticide risk assessment. In this opinion, the use of spatially explicit population models together with TK/TD modelling is proposed for six focal species. However, few population models are currently available which can be used in this context. In particular, the specific life history traits of amphibian species distinguish them from other vertebrates (e.g. different breeding and feeding habitats). Therefore, a modelling framework is proposed which can be used to simulate amphibian species in the context of ecological risk assessment. This framework is included in the existing population modelling framework POLARIS to facilitate a flexible risk assessment of various species in different landscapes.

MO174
Effects of landscape structure on PT values and population-level risk
J. Kleinmann, WSC Scientific GmbH; M. Wang, WSC Scientific GmbH / Dept Efate Modelling
In current first tier risk assessment according to EFSA (2009), PT values may be used as a refinement option. These values are mostly derived from field studies. Although these studies can be rather comprehensive and expensive, their sample sizes are often relatively small and consequently samples from different landscapes are sometimes pooled without testing the influence of landscape structure. To overcome these limitations of field studies an existing modelling framework is used to simulate PT values of various species (brown hare *Lepus europaeus*, European rabbit *Oryctolagus cunniculus*, common vole M*icrotus arvalis*) in differently structured agricultural landscapes. It is shown, that PT values strongly depend on the landscape structure and how worst-case landscapes may be chosen for field and modelling studies.

MO175
A common issue: wildlife risk assessment supported by population modelling

- a case study that investigated potential herbicide effects on common vole populations

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Environmental risk assessments (ERA) for pesticides usually require higher-tier data for the small herbivorous mammal scenario represented by the vole. Population modelling for a relevant vole species is one way to provide such data. This exemplarily case study conveys all relevant steps that were conducted for a common vole (*Microtus arvalis*) population modelling study for higher-tier ERA. The modelling study evaluated the likelihood of long-term repercussions on common vole populations due to the 2,4-D herbicide uses in grassland. It enhanced the lower-tier standard assessment as well as higher-tier field studies. The population modelling approach, its reasoning and its inclusion into the higher-tier data package was discussed with the evaluating member state authorities. The spatially-explicit individual-based population model, *eVole 3.0,* was used for simulations. It considers ecological processes such as reproduction, mortality and spatial behaviour, which are relevant for common vole population dynamics. Vole individuals in the model live in landscapes that for this study represented grassland in which herbicides were applied. The resulting foliar residues and their fate were simulated. Individuals got exposed via their diet, the assumed main exposure route, i.e. the contaminated grass within their dynamic daily home-ranges. Thus, dietary exposure was estimated in the model following the current EFSA Birds and Mammals Guidance rationale. Individual-level toxicological effects resulted from exposure according to dose-response relationships calibrated from multi-generation rat studies. As a result, the population-level responses emerged from the fates of vole individuals, which constituted the modelled populations. Increased application rates were simulated in addition to the intended rates to demonstrate a margin of safety and to provide positive controls. Finally, the impact of year-on-year herbicide applications in grassland on common vole population density was assessed by comparing treatment simulations to control simulations. Treatment-related population-level effects were evaluated and, where relevant, the time needed to achieve population recovery following the cessation of herbicide applications was determined. The study design and results reporting were guided by EFSA scientific opinions on good modelling practice and on recovery in ERA.

MO176

Can individual-based models with reduced complexity predict rotifer population dynamics under copper stress?

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The extrapolation from individual-level endpoints to the population level remains a critical issue in the ecological risk assessment of chemicals and metals in particular. Population modelling is regarded as a promising tool to bridge this gap. Individual based models based on dynamic energy budget theory (DEB-IBM) in particular are proposed as the “missing link” between the individual and the population level. Model complexity is however a hurdle for their implementation as regulatory tools because this limits the interpretability and transparency of model predictions when evaluating the model. In the current study, a DEB-IBM with reduced complexity (DEBkiss-IBM) was used to simulate effects of copper on populations of the rotifer *Brachionus calyciflorus*. DEBkiss follows a strict set of assumptions that reduce the complexity of a full DEB model e.g. by omission of maturity and reserve dynamics. The DEBkiss-IBM was calibrated at the individual-level and validated using independent datasets. Lethal effects were simulated using the General Unified Threshold model for Survival (GUTS). Sub-lethal effects on reproduction were modelled following the DEBtox principles and the physiological mode of action was determined. Both models were evaluated using independent datasets containing survival and reproduction data under copper stress. As a final validation step, population dynamics were simulated under different copper stress conditions and validated with data from a 28-days population experiment. The DEBkiss-IBM was able to succesfully predict rotifer population dynamics under copper stress. For species where the DEBkiss assumptions are valid e.g. species with a short life cycle that lack a significant reserve compartment, DEBkiss-IBMs could be used as a valid alternative to more complex DEB-IBMs, possibly easing their regulatory acceptance and use.

MO177

Population modelling to assess the effects of a copper pesticide on rainbow trout (Oncorhynchus mykiss)

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Currently, ecological risk assessment at lower tiers is mostly based on laboratory toxicity tests that measure the effect of pesticides on individual-level endpoints. Mortality, dry weight, biomass, fecundity and hatchability are individual-level endpoints that are often measured in fish toxicity tests. However, individual-level endpoints do not necessarily translate directly to a similar effect at the population level and guidelines based on individual-level endpoints might thus be inadequate for the protection of species at population level. Population models can help to overcome this problem. Therefore, the goal of this study was to extrapolate the effects of a Copper hydroxide WP pesticide on rainbow trout (*Oncorhynchus mykiss*) from a toxicity test to more realistic exposure scenarios, using a population model. To estimate population level effects, an existing individual-based model for brown trout, inSTREAM-Gen was adapted and extended with a toxicity component. Pesticide effects on survival were incorporated in the model using toxicokinetic-toxicodynamic models e.g. the General Unified Threshold for Survival (GUTS) model. These toxicity models are specifically designed to cope with time-varying exposure concentration, which are typical for pesticides. The population model was used to predict population dynamics over a multi-year simulation period and to assess how a realistic exposure to the pesticide is expected to affect this population. To this end, different exposure scenarios were selected where e.g. the number of pesticide applications and the application period were altered. Effect concentrations were derived for a selection of population-level endpoints (e.g. relative population size, probability of persistence) and critically compared with the effect concentrations derived for the individual level. The use of population models and realistic exposure scenarios offers a chance to extrapolate the effects of laboratory toxicity tests to the field based on solid ecological principles. This approach can serve as an intermediate tier tool that utilizes the results of lower tier tests and avoids the costs associated with higher tier multi-species tests.

MO178

Population level risk assessment of copper toxicity to rainbow trout (Oncorhynchus mykiss)

S.D. Janssen, Ghent University (UGent) / Vakgroep Dierwetenschappen en Aquatische Ecologie; K. Viaene, Arche consulting; K. De Schamphelaere, Ghent University (UGent) / Environmental Toxicology

Copper is an essential trace element for organisms. However too high copper concentrations will have toxic effects on organisms. It is therefore important to address the toxic effect of copper in the most accurate way possible. Nowadays, ecological risk assessment of metals is mostly based on laboratory toxicity test that measure the effect of a metal concentration on individual-level endpoints. However, it does not consider how this eventually will affect the population. Different toxic effects will happen simultaneously and may strengthen the effect of a toxicant on population level. On the other hand, population level processes as density dependence may buffer the toxicity effects on population level. Population models can be a promising tool assess the effects of a toxicant on the population level and thereby overcome these problems. Therefore, the aim of this study was to investigate population level effects of copper toxicity to rainbow trout (*Oncorhynchus mykiss*) using a population model. The study aimed to incorporate the bioavailability of copper in the population model. Another focus of the study was to incorporate different sensitivity levels to copper of the different life stages of rainbow trout. To model copper toxicity on the population level of rainbow trout, an already existing individual based model (IBM) for brown trout, inSTREAM-Gen was adapted and extended with a toxicity component. Based on individual-level toxicity data, copper effects on growth and survival of different life stages were included. Survival effects were modelled using the General Unified Threshold for Survival model based on free copper ions. The population model was used to predict relative population sizes over a 10-year simulation period. These predictions were then used to determine population level ECx values based on population density and the probability of persistence of the population after 10 years. \n

MO179

Multi-Model Framework for Assessing Pesticide Risk across Ecological Hierarchies

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Population and ecosystem models have a long history of development and use in basic research and resource management. More recently, guidance and case studies have been developed for their use in regulatory risk assessments for legacy chemicals, consumer products, and pesticides. Building on earlier multi-model approaches, we present a 3-phase framework for calibrating population models to the results of pesticide laboratory toxicity tests, then extending to field-population modeling and ecosystem modelling. The modelling framework takes advantage of the plethora of laboratory data created for pesticide registration, and facilitates evaluation of multiple scenarios incorporating environmental realism. The first phase applies population models to laboratory test results to calibrate the models and explore population responses under constant environmental conditions and relatively short simulation periods (e.g., days to weeks). The second phase extends the modelling to assess the potential effects of pesticides on populations subjected to long-term environmental regimes (e.g., time series of water temperature, solar radiation, nutrient and water inflows/outflows, and organism immigration/emigration) and pesticide exposure profiles developed using methods from FOCUS guidance. The third phase of the framework applies ecosystem modelling informed by the calibrations of population models from the two prior phases. AQUATOX (v3.2 beta) is an USEPA approved modelling software used in the third phase of this framework. Single species- and community-level effects (both direct and indirect) resulting from a combination of time-variable pesticide exposure and environmental forcing are evaluated in AQUATOX models for agricultural ditches, ponds, and streams. In addition to improving ecological realism, the multi-model framework can also be used to better inform the design of lab and field experiments and perhaps lessen dependence on them. The multi-model framework is also readily adaptable, in that the choice of a particular model or set of models within one level of an ecological hierarchy (population to community to ecosystem/landscape) may differ across risk assessments and chemicals depending on the non-target species defined in the problem formulation.

MO180

Expected environmental imact from chemicals can be modeled with sufficient accuracy and precision

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Expected impacts of chemical substances on aquatic ecosystems can be described and predicted well by means of toxic pressure modeling. Uncertainty in model output has remained an issue. Recent studies in the framework of the EU project SOLUTIONS have convincingly demonstrated that uncertainties and variabilities of model parameters, and of modeling assumptions can be adequately dealt with in toxic pressure modeling. It has been shown that state-of-art environmental impact modeling by means of toxic pressure calculation can be carried out for all chemicals that are currently used in the EU. \n This paper focuses at the newly gained insight that toxic pressure modeling using the Van Straalen-Aldenberg convolution integral (i) intrinsically accounts for variances in model output that arise from uncertainty and variability, and (ii) intrinsically deals with simultaneous presence different chemicals. Results from earlier studies are revisited. \n These results are used to demonstrate how increased parameter uncertainty and variability automatically yield greater probability that exposure concentrations exceed critical effect concentrations, and how additive mixture toxic pressure automatically results from integration across all possible values of toxicologically standardized concentrations. It has become evident that the Van Straalen-Aldenberg integral yields uncertainty- and mixture-inclusive modeling results. These results also explain the often observed phenomenon of having Pareto-distributed toxic impacts: overall (mixture) toxic pressure originates from a few chemicals only, namely those in right tail of the concentration distribution. For C_W exceeding *HC50*, Pareto ratios are found to be generally (much) greater than the often-quoted ‘80/20 rule’, namely > 95/5.\nThis means that toxic pressures of chemicals in the environment, even those calculated under relatively great uncertainty and/or variability, can serve well as a scientific basis for regulatory decision making, notably for the REACH regulation, where, in order to allow a chemical on the market, ‘safe use of chemicals’ needs to be demonstrated by registrants. This can be understood entirely from the sensitivities of the Van Straalen-Aldenberg convolution integral to uncertainties and variabilities of model parameters.

MO181

Assessment factor and SSD methods: which one provides more protective threshold for the ecosystem against adverse effects of toxicants?

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In general, a predicted no effect concentration (PNEC) is extrapolated by given information of toxicity tests in two ways: applying assessment factor (AF) and using species sensitivity distribution (SSD). AF-method is a conventional way which derives PNEC by dividing the lowest no observed effect concentration (NOEC) by AF. SSD-method, which is increasingly used in EU and USA, firstly estimates the mean and variance parameters based on given NOECs and derives PNEC by dividing a concentration leading to a protection of 95% biological species (HC5) by AF (1 - 5). To date, little is known about which one is more reliable to achieve a protection goal in ecological risk assessments. In this study, we compared which method could more likely to achieve the protection goal. We hypothetically assumed a toxicity distribution for species, and carried out random sampling from the distribution. This process corresponds to the toxicity tests supposing no toxic information about the species in advance, and the sampled values correspond to NOECs. The protection goal is defined as HC5 of the hypothetically assumed distribution. PNECs are determined by dividing the lowest NOEC by AF (fixed at 10 in our study) in AF-method. In SSD-method, HC5s are firstly estimated based on given randomly sampled NOECs and then divided by AF to derive PNEC. The AF is not a fixed value, but is contrived to vary depending on variations in species sensitivity. In both methods, determinations of PNECs are carried out for 10,000 times, and the occurrence of PNECs which are higher than HC5 (this corresponds to the failure of PNEC determination) is counted to compute the probability of failure to achieve the protection goal. In AF-method, the probability of failure is very low when the species sensitivity is low, but high when the sensitivity is high. Contrary, the probability of failure in SSD-methods is almost constant for any variations in sensitivity, and hence the probability of failure in SSD-method is higher than that in AF-method when variation in sensitivity is low, but is lower when the variation is higher. Our results imply that there are no silver bullets; one is superior than the other at a condition, but inferior in the other case. It should be recognized that the variance of the SSD is the key factor for the deriving PNEC.

MO182

SETAC Effect Modeling Interest Group

A. Focks, Wageningen Environmental Research / Environmental Risk Assessment Team

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4D-Risk Assessment (P)

MO183

Flooded Rice Pesticide Risk Assessment: the "orphan" cereal in current Guidance Documents

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European Pesticide Registration requires a Risk Assessment for non-target organisms which has to be performed according to EU Regulation (Reg. 1107/2009 EC). Through the years European Authorities developed Guidance Documents (GD) for risk assessment considering exposure scenarios for the required organisms typical for terrestrial crops. GDs do not include scenarios for pesticide applications on rice: paddy is characterized by flooded cultivation conditions, typical of semi-aquatic environments and wetlands, representative of a unique exposure scenario, not comparable to the terrestrial ones. The current and available GD could be considered not adequate and sufficiently representative to describe and evaluate the risk in rice paddy during flooded periods. Aim of this work is to identify the main critical issues raising from the GD application for the mandatory categories of organisms: terrestrial vertebrates, aquatic organisms, non-target arthropods, soil organisms, non-target terrestrial plants. A selection of possible exposure scenarios will be suggested for each group of organisms to define an appropriate approach for the development of a reserved risk assessment for pesticide applications in rice cultivation.

MO184

Identification of terrestrial vertebrate species in rice paddy

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Pesticide registration in EU (Reg. 1107/2009 EC) requires appropriate risk assessment for non-target organisms including birds and mammals. The European Food Safety Authority developed a Guidance Document (GD) which doesn’t include scenarios for pesticide applications on rice; nowadays risk assessment is generally performed considering rice as the other cereals. Rice paddy is characterized by flooded cultivation conditions, typical of semi-aquatic environments and wetlands, representative of a specific exposure scenario. The avian and mammal community in rice area is composed by species and feeding guilds which are different from the one of a general cereal exposure scenario. The present work has considered the rice area growing in the North of Italy (Lombardy Region). For birds, data from monitoring program were processed through a Generalized Additive Model (GAM) to draw probability presence maps, while for mammals information related to species autoecology were used to draw suitability maps. The obtained maps were then integrated with land use maps to delineate the rice cultivation area (GIS approach). The aim of this work is to identify focal species, in relation to the specific feeding guilds, that will be proposed for the lower tiers of the ecotoxicological rice paddy Risk Assessment and suggested as potential model for the Southern European Zone involved in rice cultivation.

MO185

Comparison of endpoints from laboratory test and mesocosm studies for risk assessment of pesticides

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For the aquatic risk assessment of plant protection products (PPPs) a tiered approach is used to estimate effects on non-target taxa in the natural environment. To extrapolate from single species laboratory studies (tier 1) to the effects expected in the 'real world', assessment factors (AF) are applied to the endpoints to achieve a regulatory acceptable concentration (RAC). In the regulatory framework more realism can be introduced by performing higher tier (tier 3) experiments, e.g. mesocosms are used as a proxy for natural surface water bodies. The tiered system is designed so that tier 1 is protective of tier 3, and by moving to higher tiers experiments progress from controlled systems in the laboratory to less controlled and realistic environments. This approach has been criticised as unrealistic at tier 1 and accompanied by high uncertainty at higher tiers and thus as either overprotective or not protective enough. The move from tier 1 to tier 3 can be viewed as an experimental extrapolation and the increased realism is reflected in the EFSA Aquatic Guidance by the use of lower AF's to calculate the ecological threshold option (ETO)-RAC. Here, we compare the endpoints derived from mesocosm studies to those determined in standard tests for the same compounds. Using this approach, we can not only assess if the tier 1 assessments are protective for aquatic ecosystems, but also explore how the introduction of dynamic and complex structures affect endpoints. To facilitate extrapolation (with the ultimate aim to extrapolate to the natural environment), we a) compare the RAC between the tiers by the means of case studies for insecticides, herbicides and fungicides; b) investigate similarities and differences of community responses within the groups of plant protection products; and c) explore future steps. The comparison of the RACs between tiers shows that laboratory study endpoints are more conservative than mesocosm endpoints, however, there is a risk that sensitive organisms are overlooked. Investigating community responses within each discussed PPP group shows that crustaceans are among the most sensitive species, and a decrease in crustaceans results in increases in competitive groups less affected by exposure. Knowledge of these community responses can help to inform models and extrapolate effects to the natural environment, but can also enable standard laboratory studies to be customised to be more relevant to the effects of certain compound groups.

MO186

Validation of a stream ecosystem model: Available data - challenges - future perspectives

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Ecosystem models are powerful tools for predicting species population dynamics for different environmental scenarios under consideration of natural and anthropogenic stressors. In developing such complex models, simplified assumptions have to be made about the relevant processes to be represented in the models. While effect models such as the TKTD model GUTS are at least conceptually easy to parametrize, it is much more effort to parameterize the sensitive ecosystem properties, such as e.g. the food availability for the various feeding types of macroinvertebrates. However, for all models it should be demonstrated that the model is able to reproduce relevant ecological scenarios with sufficiently high accuracy, what is known as "model testing" or "validation". Furthermore in this context it is of importance what type of data is available and of suitable quality for this step. One possibility is to explore macroinvertebrate dynamics over time which is available from monitoring data. In this study the concept of validation of an aquatic stream ecosystem model (STREAMcom) will be demonstrated. The STREAMcom simulates the population dynamics depending on spatial explicit habitat maps as well as temporal information on abiotic factors like temperature, food availability and chemical exposure. Additionally functional trait data bases, dynamic energy budget based population models and process based effect models are included in the STREAMcom model. The usability of available monitoring data for small streams as data set for validation of macroinvertebrate population dynamics in the model as well as associated challenges and future strategies for model applications will be discussed.

MO187

Predicting the sensitivity of invertebrate assemblages to toxic chemicals

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Biodiversity varies across natural ecosystems, with each being composed of different species assemblages. Yet current environmental risk assessments (ERAs) for chemical exposure are associated with two issues around predicting the sensitivity of natural ecosystems. Firstly, ERAs are generic, and a single regulatory acceptable concentration is utilised across Europe. This is irrespective of spatial variation in sensitivity that arises from differences in species composition. Secondly, standard laboratory toxicity tests for ERAs are only performed on a limited selection of standard test species. Consequently, the sensitivity for most species in natural ecosystems is also unknown. Combined, these issues mean that the spatial variation in predictions and the data with which to calculate such predictions are unknown. To address these issues, the hierarchical species sensitivity distribution (hSSD) approach has been taken to predict a toxicity threshold value. As the hSSD method predicts sensitivity for specified assemblages of species, varying assemblage composition can reflect different natural ecosystems. The hSSD method utilises phylogenetic relatedness and known toxicity data to predict toxicity to a chemical for each species of an assemblage, then integrates these predictions into species sensitivity distributions. The hSSD method was applied to a dataset of macroinvertebrate assemblages from 835 minimally impacted river sites across the UK to quantify variation in the hazard concentration affecting 5% of species (HC_5) derived from hSSD curves. Six chemicals of multiple types and modes of action were tested, including pesticides, down-the-drain chemicals and biocides. The predicted HC_5 values exhibited patterns based on different chemical types. Assemblage sensitivity was also related to the river typology of the sites. As the species present at each site depended on the river typology, the effects observed reflect the influence of species composition on assemblage sensitivity.

MO188

Relevance of systematically screening scientific literature for emerging contaminants - a case study in the Netherlands

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Contaminants in the aquatic environment pose a potential threat to ecosystems and to humans, especially when water resources are used for drinking water production or recreation. Climatological, social and demographic changes as well as the increasing sensitivity of analytical techniques, will result in the augmented detection of contaminants. Thus, effective risk governance of contaminants in the aquatic environment is and will remain very important in order to protect human and environmental health. Here, emerging contaminants are defined as chemical contaminants that pose a new or increased threat to public health or the environment. In recent research, we have shown that the current risk governance of emerging contaminants in the aquatic environment could be improved by more timely identification of contaminants. Therefore, we propose a methodology using scientific literature text mining for picking up the first signal of the detection of a chemical contaminant in the aquatic environment. This first signal is a scientific article that reports the first identification of chemical contaminants in the aquatic environment. Once the first signals are determined, an assessment of the identified signals can be performed to elucidate the national relevance of the contaminant. A longlist of potential contaminants was screened using criteria such as 'source presence', 'persistence in the environment' and (when available) potential 'toxicity'. The resulting short-list was then distributed amongst Dutch authorities and drinking water companies in order to evaluate non-target screening data on the presence of these contaminants. Consequently, a monitoring study for the contaminants was carried out at relevant locations throughout the Netherlands. The locations were chosen taking into account for example specific usage information of the contaminant (e.g. for pharmaceuticals), potential production locations (e.g. for industrial contaminants), hydrological information of the receiving waters and the catchment and proximity to a drinking water intake point. In this study, an assessment of the national relevance of the results of an automated textual screening of the scientific literature was carried out for the Netherlands. The Dutch case study showed that systematic screening of the existing universal scientific literature and determining local relevance can give new insights into aquatic contaminants.

MO189

Putting the TKTD modelling Scientific Opinion of EFSA into practice – the GUTS model

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The European Food Safety Authority (EFSA) published a Scientific Opinion in toxicokinetic/toxicodynamic (TKTD) effect models for regulatory risk assessments for aquatic organisms in 2018. There, the EFSA Panel on Plant Protection Products and their Residues (PPR) scientific committee concluded that a TKTD model for survival (i.e., the General Unified Threshold models of Survival – GUTS) is ready for use. This is a major step for the regulatory community since it provides clear guidance how to apply as well as for how to evaluate TKTD models for the registration of chemicals in Europe. Furthermore, this Scientific Opinion identifies existing gaps and further regulatory and research needs in existing TKTD models. Although the Scientific Opinion was devoted to the aquatic environment, the linking of GUTS to other areas of the risk assessment is possible. Here we present a case study employing GUTS for assessing the toxicity of a fungicide on honeybees. The objective of this presentation is to identify the expected deviations from the guiding principles of the EFSA Opinion if GUTS is used for honeybees. These deviations will be discussed with respect to the robustness and reliability of GUTS beyond aquatic risk assessment.

MO190

Comparison of Ecotoxicological Endpoints: National Risk Assessments versus Commercially Available Databases

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To calculate indicators of environmental risks for multiple co-occurring pesticides at landscape scales, researchers mostly rely on commercially available databases, which give information on ecotoxicological endpoints. A full coverage of all pesticides and the comparability of study types is important to get suitable endpoints for the task. Databases such as the Pesticide Properties Database (PPDB) are helpful in this context. Using the PPDB for our own purposes, we occasionally came across differences between endpoints from this database and those used for registration of plant protection products by Swiss national authorities. To better understand these differences, a comparison between the endpoints reported in the PPDB and the endpoints used for the registration of plant protection products was conducted. In the majority of cases the endpoints were similar. However, in some cases significant differences even by more than a factor of 10'000 were found. Possible reasons behind largely differing endpoints were investigated and endpoints to be used for risk indicators for co-occurring pesticides are suggested. The comparison illustrates the importance of challenging endpoints provided by databases, and integrating the experience and knowledge of national authorities on single active ingredients to improve indicators of environmental risks.

Criteria and Methods for Identifying Chemicals of Greatest Concern (P)

MO191

TOXMIC: TOXicants monitoring chamber for MICrocontaminants

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There is no doubt that water is a necessary resource for life and that human activity depends on water quality and availability. In spite of the efforts made in the last decades for improving waste water treatment and for the implementation of the Water Framework Directive, water pollution is still a main cause of water quality deterioration. Ecological quality standards for single substances and for mixtures have been established but uncontrolled release of chemicals in waters will continue until the water diagnosis capacity will be incremented. In particular, in EU about 71000 Waste Water Treatment Plant outflows need to be constantly checked, especially during water scarcity periods and the traditional monitoring, based on grab samples, is not economically affordable. The aim of TOXMIC project is to deliver an innovative low-cost in-situ monitoring device able to check in continuous water quality of rivers and capable of being widely taken up by WWTPs and water agencies, ensuring in this way a positive impact on the ecosystem and human health and on business growth. The monitoring chamber has been developed in laboratory and tested in selected sites in Catalan rivers in collaboration with the Catalan water Agency. The chamber combines two existing techniques with an innovative integrated sensor: 1. Passive sampling Polar Organic Chemical Integrative Samplers POCIS, for polar organic compounds, and Diffusive Gradient in Thin film DGT, for trace metals; 2. Pulse-amplitude modulated PAM fluorometry applied to river biofilm structural and functional indicators; 3. Micro-PAM sensor for data transmission in continuous. A local reference is obtained with a reference chamber where river water is filtered by charcoal. The link between contaminants and effects on biofilm is analysed using advanced modelling techniques (mixture effects models and classification tools).

MO192

Do we need new chemical assessment criteria? - Consideration of the recent proposal for application of persistent, mobile and toxic (PMT) criteria.

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Recently, there have been discussions surrounding new screening criteria based on a chemical having inherent properties of being persistent, mobile and toxic (PMT) or very persistent and very mobile (vPvM). The European Chemicals Agency (ECHA) has also received a proposal to identify a potential Substance of Very High Concern (SVHC) under its regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) based on being vPvM, which was purported to be of an equivalent level of concern to already established criteria for persistent, bioaccumulative and toxic (PBT) substances, and very persistent and very bioaccumulative (vPvB) substances. The purpose of the proposed assessment criteria was the protection of drinking water sources. The utility of a new set of assessment criteria for identifying chemicals of greatest concern was examined in the context of existing chemical management regulations in Europe and North America. In addition, the historical development of assessment criteria was considered particularly with regard to the assessment tools at the disposal of regulatory authorities in those geographies at the time of their development. Finally, given the demands on both regulators and the regulated community in the implementation of a new set of assessment criteria, recommendations are provided regarding appropriate steps for its proper evaluation. \n

MO193

Version 2.0 of the Ecological Risk Classification (ERC) Approach for Prioritizing Organic Chemicals in Canada

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In 2016 Environment and Climate Change Canada (ECCC) published the Ecological Risk Classification (ERC), a computational approach used to re-prioritize 640 organic chemicals originally prioritized in 2006 as persistent or bioaccumulative and inherently toxic. The ERC is a risk-based approach developed to optimize the use of new approach methods (NAMs) in ecotoxicology and novel exposure descriptors for chemical prioritization. The second version of the ERC (ERC2) is under development by ECCC for the prioritization of approximately 12200 organic chemicals on the DSL not prioritized in 2006 as BT or PT chemicals. It will provide ECCC with one approach (substance-by-substance) for determining chemicals of most concern for post 2020 chemical's management work planning. The ERC approaches establish chemical profiles for hazard and exposure. Each profile contains several descriptors that define both the toxicological and exposure spaces used for classification. Logic rules developed for these spaces classify organic chemicals for hazard, exposure and ultimately risk at three levels. Overall risk is determined using a matrix approach. As with ERC1, ERC2 remains an evidence driven rule-based prioritization system, but expands on the toxicological and exposures space from ERC1. ERC2 also addresses key areas of uncertainty identified in ERC1 by better accounting for model domain of applicability and improving on the *consensus* within and among*in silico, in chemico, in vitro, in vivo and in situ* data to classify hazard, exposure and risk. ERC2 also provides an assessment of confidence at all levels of data and for final, hazard, exposure and risk classification based on the degree of data consensus. ERC2 also employs the AOP concept to organize hazard data to provide plausible causal relationships that link chemistry with biology (i.e., mechanistic reasoning for adverse outcomes). Biological read-across is used to incorporate data from laboratory mammalian studies and models given the high degree of conservation of pathways across species. In addition to risk classification of 12200 organic substances, ERC2 will also provide ECCC with data to respond to various regulatory questions such as identification of potential endocrine active substances, regrettable alternatives, high hazard or exposure concerns, potential research and monitoring priorities, etc.).

MO194

The new Microimpedance Sensor System© (MSS) for online biosensing of water quality with microcrustaceans

A. Gerhardt, LimCo International GmbH

Online biomonitors can be interpreted as whole organism biosensors: a sensitive behavioural or physiological response of a sentinel species is used as biological warning system for pollution stress. Continuous biological monitoring of the toxic potential generated by pollutant cocktails in surface waters is already established, e.g. along large transboundary rivers in Europe. Frequently used animal test species for online biosensing have been *D. magna*, mussels and *Gammarus* spp. Based on the Multispecies Freshwater Biomonitor© (MFB) for animal sizes of > 5 mm, we developed the Microimpedance Sensor System© (MSS) to continuously record the quantitative behavioural pattern of small crustaceans and young life stages of epigean and hypogean invertebrates (size ca. 1 mm). These tiny organisms are often additionally transparent, hence difficult to observe with optical tools. Interstitial and sub-surface habitats are inhabitated predominantly by small organisms, such as copepods. These species have a high potential as toxicological test species and online biomonitors of groundwater and drinking water, provided they are more sensitive, easier to handle and generate longer stand-alone times of online biomonitors, to sense also chronic pollutant stress. The MSS has successfully been tested and calibrated with *Eucyclops serrulatus* (Copepoda). *E. serrulatus* detected acute pulses of copper and nitrate. Ease of culture and handling additionally opt for *E. serrulatus* as new indicator in biomonitors.

MO195

WATER TREATMENT PROCESSES AND THE POTENTIAL FOR SUBSTANCES OF CONCERN TO ARISE FROM CROP PRODUCTION PRODUCTS

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In 2015, a new requirement was introduced for active substance submissions (including submissions for renewal) in the EU to consider the effect of water treatment processes on pesticides in drinking water. The requirement forms part of the approval criteria for active substances as detailed in Article 4.3(b) of Regulation (EC) No 1107/2009 where they: *...shall have no harmful effect on human health, or animal health, directly or through drinking water (taking into account substances resulting from water treatment), food, feed or air, or consequences in the workplace or through other indirect effects, or on groundwater.* In the absence of any guidance, or indeed information on the actual water treatment processes to be considered, this review provides an overview of current water treatment technologies, the principles behind them and the common side reactions often encountered. This information is then used to suggest potential unwanted harmful by-products that could arise from purification of abstracted water containing pesticide residues. Finally, previous submissions are evaluated to see how applicants have approached Article 4.3(b) to date, and whether or not a consistent approach has been adopted.

MO196

Comparing Mode of Action (MoA) classification using body residues, membrane concentrations and chemical activity for chemical prioritization

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In 2016, Environment and Climate Change Canada (ECCC) released the first version of an IATA-like Ecological Risk Classification system (ERC1) for prioritizing the organic substances on the Canadian Domestic Substances List (DSL) remaining to be assessed before 2020. One of the key considerations for assessing toxicity in ERC1 is a Mode of Action (MoA) classification which aims to distinguish between baseline toxicants ("narcotics") and specifically-acting toxicants in a given test organism/receptor. As part of the MoA classification for aquatic organisms (fish), ERC1 used an estimate of the Critical Body Residue (CBR) corresponding to an empirical or QSAR-based lethal concentration (LC50). Two alternative/complementary metrics for MoA classification that have been advanced recently are i) Critical Membrane Concentration (CMC) and ii) (lethal) chemical activity (La50). At the screening level, the CBR, CMC and La50 approaches rely on different physical-chemical properties to "translate" the LC50, namely octanol-water partitioning (K_{OW}), membrane-water partitioning (K_{MW}) and water solubility respectively. The main objective of this study was to generate CBRs, CMCs and La50s for a set of 929 neutral organic chemicals with empirical LC50 data and compare the MoA classifications across the three metrics and to the MOA classification based on another approach applied in ERC1 (structural alerts). Complete agreement in the screening level MoA classification using CBR, CMC and La50 was observed for 719 of 929 chemicals (599 baseline toxicants, 120 specifically-acting). Complete agreement in the screening level MoA classification using CBR, CMC, La50 and structural alerts was observed for 585 of 929 chemicals (522 baseline toxicants, 63 specifically-acting). Explanations for the apparent discrepancies include uncertainties in property measurements/estimates, problematic toxicity data (e.g., LC50s greater than water solubility) and the domains of applicability for all approaches (including MoAs based on structural alerts). The findings of this exercise will be used to inform version 2.0 of the ERC, which is being developed as one approach to determine chemical priorities for post 2020 chemicals management in Canada.

MO197

Biological module bioluminescent biosensor for environmental risk assessment

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Bioluminescent enzyme systems based on bacterial luciferases offer a unique and general tool for assays of the many analytes in the environment, research and clinical laboratories and other fields. The goal of the study is to develop a biological module bioluminescent biosensor based on bioluminescent enzyme systems for environmental risk assessment provided an alarm detection and quantitative measurement of integral toxicity levels caused by chemical threats. The biological module for the enzyme-based biosensors includes co-immobilized into dried starch gel bacterial coupled enzymatic system: NADH:FMN-oxidoreductase and luciferase and their substrates (mirystic aldehyde and NADH). The bioluminescent biosensor controls the effect of the sum of toxicants. It was shown that starch gel has some stabilizing effect on the enzymes of bioluminescent bacteria. The biomodule maintains the high enzymatic activity in a wide range of temperatures, pH and ion concentration. The effect of toxicants (phenols, quinones and heavy metal salts) on immobilized coupled enzyme system NADH:FMN-oxidoreductase – luciferase has been analyzed. We determined I?$_{50}$constituted 50 % of the loss of biomodule's luminescence for each of the analyzed substances. Biomodule has been shown to be more sensitive to quinones. The sensitivity of detection for naphtoquinone, benzoquinone, tolyquinone and timoquinone were less than their maximum permissible concentration.There is a good correlation between the results obtained and chemical analyses of the industrial waste waters of pulp-and-paper plant (Krasnoyarsk, Russia). Thus, co-immobilized bacterial coupled enzymatic system: NADH:FMN-oxidoreductase and luciferase and their substrates into starch gel is stable and sensitive. Bioluminescent assays based on co-immobilized bacterial coupled enzymatic system are fast (analysis time is 2-6 minutes), simple, sensitive, and accurate. The immobilized bacterial coupled enzymatic system may be use as biological module bioluminescent biosensor for environmental risk assessment was shown. The reported study was funded by Russian Foundation for Basic Research, Government of Krasnoyarsk Territory, Krasnoyarsk Regional Fund of Science, to the research project No 18-44-242003.

MO198

Detection and killing of Staphylococcus aureus by specific peptides-conjugated-vacuoles in yeast

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Staphylococcus aureus is a major human pathogen causing healthcare-associated and communitu-associated infections. In this study, we provided a novel system that is available to detect and kill *S. aureus* specifically based on the conjugation of phage display library technique and yeast vacuoles. Here, a phage clone displaying peptide able of specific binding to *S. aureus* whole cell was selected from a 12mer phage peptide library (peptide sequence: AAHRVGGFNYHM). The ability of selected phage to bind specifically with *S. aureus* was confirmed by using enzyme linked immuno assay (ELISA), and then the chosen peptide was synthesized and conjugated with the Dynabeads M-270 Carboxylic acid to demonstrate its specific binding ability with *S. aureus*. The results showed that the synthesized peptides displayed high affinity with *S. aureus* but low binding ability with other strains including gram negative and positive bacteria, such as *Salmonella* sp., *Shigella* sp., *Escherichia col*i and *Corynebacterium glutamicum*. After that, the peptide was conjugated with our yeast vacuoles, which was proved in previous studies that possess antimicrobial activity. Finally, the novel system containing specific peptides for *S. aureus* and yeast vacuoles showed the high ability to recognize and kill *S. aureus*. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No:PJ01267701)' Rural Development Administration, Republic of Korea.

MO199

An Analytical Method for Screening and Quantification of Pyrrolizidine Alkaloids-Natural Toxins in Surface Water

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Phytotoxins are natural toxins produced by plants, and they have been produced and loaded into environment in high doses. They are not distinguished as contaminants of possible concern for environment and water. Thus, environmental fate and content of such natural toxins are largely unknown. The purpose of this study to show an analytical method to screen and quantify Pyrrolizidine Alkaloids (PAs) in water and environmental samples, without requiring all corresponding standards. PAs were found in many plant species of families: Asteraceae, Boraginaceae and Fabaceae. Until now, more than 660 PAs have been found in nature. UPLC- MS/MS method developed by a Waters Acquity UPLC I-Class module, equipped with a 2.1 × 50 mm, 1.6 µm Cortecs C18 column (Waters, Milford). MS was operated on a Waters Xevo TQD MS with positive ion mode. In the method, LC mobile phase was composed of A (water + 0.01% FA) and B (acetonitrile + 0.01% FA). Gradient conditions were: 0−4 min 10% B, 7 min 20% B, 10 min 50% B, 15 min 90% B, 15−17 min 90% B. The column was equilibrated for 6 min, and the total run time is 23 min. The flow rate was 0.45 mL/min. The method is quick, robust and validated for quantification of PAs in wide range complex matrixes and environmental samples such as plant, soil and water. The fragmentation pattern of PAs used in the method to characterize product ion: for retronecine and heliotridine types *m/z* 138.09 and 120.08 and for otonecine types *m/z* 168.10 and 150.09 are selected and characterized. The LoD ranged 2-6 μg/L. For application of the method, a surface water close to Ragwort (Plant as a source of PAs) catchment in Vejle-Denmark sampled, as a result 10 toxic PAs analysed in a surface water with a concertation up to 500 μg/L. This research project is part of European Training Network–NaToxAq, which has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No.722493(NaToxAq).

MO200

An approach to systematically evaluate and utilize Molecular Initiating Events (MIE) for chemical classification

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Mode of Action (MoA) determination is an important part of understanding toxicity to aquatic organisms in the context of risk assessment and as part of regulatory schemes to enable read-across, chemical categorisation and prioritisation. With a lack of harmonisation of current chemical -fragment based approaches to be able to adequately classify the full commercial chemical space coupled with a shift towards mechanistic based approaches, there is a need to better define classification approaches. The Adverse Outcome Pathway (AOP) concept has gained significant traction as a means to interpret apical toxicity endpoints mechanistically. An AOP is a systematic approach that links molecular and biochemical interactions to individual or population responses and thus provides an opportunity to base classification schemes around this framework. A first key component in AOPs is the Molecular Initiating Event (MIE), where both chemistry and biology are integral components. There is a recognised need to evaluate the confidence of all parts of the AOP notably the MIE, Key Events (KEs) and Key Event Relationships (KERs); whilst schemes are in place, for KEs and KERs, they have not been applied to MIEs. The aim of this study was to develop a scheme for classifying chemicals in MIEs relevant to aquatic toxicity. Prior to collation of MIEs a framework was established to evaluate the 'quality' of MIEs in terms of the underlying evidence. Information on structural qualities of the MIE target; the interaction; associated chemistry; taxonomic applicability; along with the sources and the type of data (e.g. in silico, in vivo, in vitro) corresponding to MIE/MIE target of interest were documented, leading to 78 identified MIE and MIE targets. The MIEs were organised in 3 classes based on the type of interaction. This facilitated the utilisation of the chemical domain of MIE/MIE targets as building units for the chemical space per class. The chemical structural criteria for class assignment along with transparency in taxonomic applicability, data source and quality, were coded in a KNIME workflow. This approach provides the user with an informed MIE prediction that can be potentially used in the application of the AOPs to better understand chemical classification and to predict toxicity across aquatic species.

MO201

Cytotoxicity assessment of the emissions to indoor air of a CdTe quantum dot based fluorescent ink

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The fluorescent properties of CdTe quantum dots (QDs) have led to novel products and applications in the ink and pigment industry. From a life cycle perspective, the toxic effects of the emissions to indoor air associated to printing ink during the use phase might differ from those of conventional formulations which do not integrate engineered nanomaterials as CdTe QDs might be emitted. Such chemicals can, thus, be regarded as emerging contaminants for which several authors have reported toxicological effects^[1],^[2]. Within this work, a water soluble fluorescent ink containing polyethyleneglycol-coated Cadmiun Telluride (PEG-CdTe) QDs (3.2 nm, 3.6 nm with coating) at a theoretical concentration of 8 mg/mL has been selected. Such ink is a prototype of a Nano enabled Product (NEP) that has been formulated to be used in household printers. Emissions of the printing process have been characterized under different simulation scenarios. Subsequently, released particles have been evaluated in terms of their cytotoxicological potential in contrast to the solvent ink. The pulmonary cell line BEAS-2B has been selected both for *in vitro* air-liquid interface cell exposure and in submerged conditions. Preliminary results indicate that the Cd2+ concentration determines the toxicity observed.

Acknowledgements: NANOSOLUTIONS GA N 309329 QNANO – GA N INFRA-2010-262163 <br clear="all" /> [1] Su, Y. et al (2010) The cytotoxicity of CdTe quantum dots and the relative contributions from released cadmium ions and nanoparticle properties. Biomaterials 31 (18), 4829-4834. [2] Rzigalinski B.A., Strobl J.S. (2009) Cadmium-containing nanoparticles: perspectives on pharmacology and toxicology of quantum dots. Toxicol. Appl. Pharmacol. 238, 280–288.

MO202

Using the Multispecies Freshwater Biomonitor to assess the potential impact of the antidepressant Venlafaxine on the amphipod Gammarus pulex

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The Serotonin and Norepinephrine Reuptake Inhibitor (SNRI) Venlafaxine (VEN) is an antidepressant that is often prescribed to treat depression and anxiety. VEN has been frequently detected in aquatic environments where its possible effects are still widely unknown. The aim of this study was to investigate the potential impact of VEN at environmentally realistic concentrations (20 ngl^{-1}, 2 μgl^{-1} and 20 μgl^{-1}) and identify if it effects the behaviour of the freshwater amphipod *Gammarus pulex*. River water and specimens of *Gammarus pulex* were collected from a non-polluted stream in Allensbach, Germany (47°42'27.6'' N, 9°06'26.5''E) during September 2017. The organisms were acclimated for 14 days at 18°C in aerated stream water in the dark. *Gammarus pulex* were provided with *Alnus glutinosa* leaves. In order to test the effects of the antidepressant VEN a serial exposure was undertaken. Specimens of *Gammarus pulex* (n=30) were used for each concentration (20 ngl^{-1}, 2 mgl^{-1} and 20 mgl^{-1}) and a control. 5 x 250 ml glass beakers were filled with 100 ml of each VEN concentration. 6 *Gammarus pulex* were put into each beaker with a 3 cm Ø *Alnus glutinosa* leaf disc. Behavioural analyses were carried out using the Multispecies Freshwater Biomonitor (MFB).The MFB is a device that tracks and records the behavioural activity of different aquatic organisms through a quadruple impedance conversion technique. Each organism was placed in a small plastic chamber in a 4 L aquarium. The presence of electrodes inside the chamber enables an organism's movement to be recorded as a change in the electrical field. A movement generates specific frequencies which in the case of *Gammarus pulex* are from 0.5 to 2.5 Hz. *Gammarus pulex* swimming behaviour was recorded 5 times over a period of 12 days. The data was analysed with a general linear effects model and it was found that there was a significant difference in their behaviour over time (F=4.107; p=0.004) and that there was a significant different in their behaviour between treatments (F=4.364; p=0.006), but there was no interaction between treatments and time (F=1.092; p=0.372). Pairwise comparisons showed a significant difference between the lowest concentration (20 ngl^{-1}) and the control (p=0.011) and the median concentration (2 μgl^{-1}),(p=0.012). Overall, VEN does have an impact on the behaviour of *Gammarus pulex,* but this effects is not related to time.

MO203

Identification of novel PMT compounds of potential concern to water resources - The case of natural plant toxins

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Recent work has highlighted the potential risk of persistent, mobile and toxic (PMT) compounds to water resources, including drinking water. Focus is often on anthropogenic pollutants, however a large number of natural toxins, produced by plants as e.g. defense compounds against herbivores and microbes, have similar properties in terms of mobility, persistency and toxicity. Plants occur abundantly in the environment and thus even small amounts released per plant, continuously or in high rainfall events, may become substantial overall. The present project, part of the European Union ETN network 'Natural Toxins and Drinking water Quality - From Source to Tap', focuses on the identification of novel natural toxins that could leach from source (plant) to tap, and cause detrimental effects in the aquatic environment in between. In this study, we sampled 20 invasive plant species based on four non-exclusive criteria – local abundance, high distribution, proximity to water resources and likelihood of toxin content – and employed effect-directed analysis (EDA) in order to identify novel toxins of concern. Secondary metabolites were extracted with water and dosed on *Daphnia magna* as a model organism for acute toxicity and neurotoxicity. Fractionation (reverse-phase HPLC) was performed on extracts with high effect levels, and current activities focus on the identification of causative compounds in active fractions (LC-HRMS).

MO204

The FET and behavioral assays under flow-through conditions - evaluation of a biowellplate utilizing zebrafish embryos

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Whole-organism bioassays, such as the fish embryo toxicity test (FET), are often applied in biological, biomedical and ecotoxicological research. However, they are mostly performed within discontinuous conditions i.e. static or semi-static exposure regimes in small units, such as multiwell plates for prolonged incubation periods. In order to improve exposure conditions of whole-organism bioassays, we tested the performance of a biowell plate-prototype (BWP) designed for the exposure of organisms within flow-through conditions. The aim was to provide a testing system that reduces labour intensity, invasiveness to the tested organisms caused by manual exchange of the exposure medium and that improves the stability of the concentration of the tested chemical in the exposure medium as well as the oxygen concentration by a continuous flow. The prototype provides a constant exchange of the exposure medium combined with a controlled temperature within the 8 wells which have dimensions comparable to those of a 96-well plate. Prolonged FETs until 96 hours post fertilisation (hpf) utilizing zebrafish embryos (*Danio rerio*) were conducted in order to assess the general performance of the BWP and the influence of the flow-through conditions on the stability of exposure concentrations and consequential the determined effect concentrations for a set of model compounds (cadmium dichloride, ethanol, permethrin, sodium hypochlorite). Implications for testing strategies of single substances in whole organism-bioassays will be discussed. Monitoring of zebrafish embryo behaviour emerges as new, sensitive method to study adverse effects of compounds. We propose to use behavioral changes of zebrafish embryos for the detection of sudden contamination events in a water stream. The applicability of the BWP as a biosensor in environmental monitoring was evaluated utilizing assays that examine the behavioural reaction of hatched embryos (96 hpf) towards acute exposure to different compounds relevant for surface waters. In this study, the zebrafish embryos were incubated in the BWP in formulated water under flow-through conditions until 4 dpf. Subsequently, they were exposed to a set of model compounds in low concentration ranges and monitored for avoidance behaviour which results in an increased activity. This first proof of concept study evaluated the general performance of the BWP in the FET and the potential application as biosensor in environmental monitoring.

MO205

Toxicological Council and SamTox - A Swedish initiative to establish an organisation of an early warning system in order to identify new, potential chemical threats

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In order to improve the coordination between national authorities, in 2016 the Swedish government decided to establish a coordination group to deal with new and emerging chemical threats (SamTox). The Toxicological Council was formed in order to improve the systematic monitoring and use of scientific information and to provide updated and relevant information to SamTox. The Council includes representatives from Swedish governmental authorities and universities. The Toxicological Council identifies and evaluates signals of new, potential and emerging chemical risks and reports its findings to SamTox. The purpose of this organisation is to ensure a structure for the rapid and systematic transfer of information and knowledge between responsible authorities and other actors, as well as cooperation in the event of the detection of a serious chemical threat. In order to develop a methodology for the joint evaluation of signals aimed at identifying potential NERCs (New Environmental Risk Chemicals) or insufficiently managed chemical risks, three areas in which multiple signals had been compiled were selected for additional work from 2017–2018. After gathering additional data, the Toxicological Council decided to raise two areas of concern with SamTox. The first, PFAS accumulating in landfills potentially resulting in a (future) source of contamination can be regarded as a potential NERC based on new information becoming available. The second, non-decreasing exposure levels of cadmium in the general population can be regarded as a known but insufficiently managed chemical risk. The Toxicological council has established a successful working procedure in order to identify NERCs. However, identifying new potential chemicals threats require more cooperation and exchange of information at the EU level and internationally. The presentation is a step to initiate such cooperation.

Difficult to Test Substances and Wastes: Regulatory Status, Testing Challenges, Interpretation of Data and Fulfilling Information Requirements (P)

MO206

Testing poorly water-soluble test chemicals according to the updated OECD Guidance Document 23 on aqueous-phase aquatic toxicity testing of difficult test chemicals

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Aquatic toxicity testing of poorly water soluble test chemicals presents unique challenges as it may be difficult to create dosing solutions and to maintain constant aqueous concentrations of these chemicals in exposure systems. Solvents are sometimes employed to facilitate dissolution of poorly water soluble test chemicals; however, the use of solvents may have an impact on the study results, particularly in chronic studies. Furthermore, the use of solvents increases the number of animals used per test by up to 14-25% depending on the study, because an additional solvent control group is recommended by the Test Guidelines. For these reasons, efforts are made to avoid the use of solvents in aquatic toxicity tests wherever possible. In recent years, new methods have become available for aquatic toxicity testing of chemicals considered to be difficult-to-test, including poorly/sparingly water-soluble. Therefore, through the Organisation for Economic Cooperation and Development (OECD) Test Guidelines Programme, the European Commission, United States, and the International Council on Animal Protection in OECD Programmes worked with leading experts to update OECD Guidance Document (GD) 23 on aqueous-phase aquatic toxicity testing of difficult test chemicals. The GD was expanded to discuss the state-of-the science approaches for testing a broad range of difficult-to-test test chemicals including poorly/sparingly water-soluble chemicals and was adopted in 2018. This poster describes state-of-the-art approaches for testing poorly or sparingly water-soluble chemicals including additional guidance on creating exposure systems using direct addition, the use of generator systems including saturator columns and passive dosing as well as flow-through exposure systems. For illustration, this poster compares water solubility and saturation concentrations in different media from studies run using saturation columns, liquid-liquid saturators, solid-liquid saturators, and passive dosing. The updated GD 23 will help government agencies, industry, and contract research organisations to conduct valid and reliable aquatic toxicity studies on difficult-to-test chemicals while minimising both the number of animals used and the need to repeat studies. *The views, conclusions and recommendations are those of the authors and do not necessarily represent the policies or positions of the organisations to which the authors are affiliated.*

MO207

Help, we've measured the wrong active!

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When testing mixed active substance plant protection products in aquatic toxicity studies at least one active substance needs to be measured in order to confirm dosing. Often only one of the active substances has been measured which can make it difficult or impossible to use the study in an aquatic risk assessment according to EFSA (2013). In order to conduct a mixture assessment it is first necessary to determine whether the plant protection product is higher, lower or of similar toxicity than would be predicted by the active substances based on additive toxicity. This is done by calculating the model deviation ratio (MDR) using the calculated and measured toxicity. In order to do this it is important that the product and active substance studies are equivalent, which means the concentrations in the studies should be known or at least confidently predicted. When an active substance is known to be stable in water, confirming the targeted concentration was achieved might be enough for this, but where it is unstable it is necessary to have measurements of that active substance over time. This poster explores what can be done in a variety of situations if all active substances in a formulation study have not been measured at least at the beginning and end of the study. There is a particular focus on fish because of the need to avoid additional vertebrate testing unless it is essential. A risk assessment method is presented where only initial measurements are available and the study is on fish, to allow the use of an old study that doesn't meet modern requirements for test substance analysis. Proposals are also made for deciding which active substance is more important to be measured if only one has been and how these endpoints can potentially be used in the risk assessment. The decision scheme presented can be used both by people conducting studies to help them decide whether all active substances need to be measured and by regulatory authorities when deciding if and how a study can be used for the risk assessment.

MO208

How to assess the aquatic toxicity of cationic polymers?

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Cationic polymers are widely used across the world. They are large, charged surface-active compounds and since they are polymers they are currently exempt from the REACH-framework. The exemption is based on the assumption that polymers due to their large size will be toxic and thereby not pose any risk to the aquatic environment. However, reduced registration requirements for polymers may lack scientific justification. Scientifically-based methods to support assessments and registration will need to be established in the future for these compounds. Cationic polymers bind to negatively charged surfaces, such as the surface of an organism or certain forms of aquatic organic substances. The bound complex may not cause direct toxicity but can indirectly harm or impair aquatic organisms. This mode of "toxicity" challenges our understanding of what causes toxicity and how we describe, test and model environmental interactions. When dealing with cationic polymers known effect-predictors as hydrophobicity may not describe physical-chemical influences on toxicity. Instead, alternative physicochemical properties as charge-density, molecular weight, polydispersity and viscosity may be more suitable to predict effects. This research will investigate how to describe and assess the aquatic toxicity of cationic polymers in a realistic way that would assist future aquatic environmental risk assessment (ERA) of these compounds. Our initial focus is on four model cationic polymers that are selected due to their representative differences in physicochemical properties: Polyquaternium-6, -7, -10 and -16. We will present our plan for this CEFIC LRI project, as well as our current findings: main points from a literature review and screening results of the acute aquatic toxicity of algae, daphnia and bacteria caused by various Polyquaternium-10's. The results are determined by tests following the guidelines ISO 11348-3, OECD 201 and OECD 202 to represent the current frame of acute toxicity testing in ERA. The results will give an indication of what physicochemical properties are driving the toxicity but also point toward current deficiencies when using standard tests to describe the effect of what could be called non-standard molecules. By discussing the results, we will present how adjustments to the current test guidelines, of e.g. the test water, will make the description of the acute aquatic toxicity realistic and applicable for modelling and ERA in a regulatory setting.

MO209

PBT Testing Strategies for Difficult Substances under REACH

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Suspected PBT (persistent, bioaccumulative, toxic) substances are often difficult to test in aqueous systems due to high lipophilicity and low water solubility. In addition, many are adsorptive, leading to problems such as adsorption to glassware and formation of NER (non-extractable residues). PBT testing and assessment of multi-constituent and UVCB (unknown or variable composition, complex reaction products or of biological materials) substances can create additional problems when deciding on the most appropriate test material and testing strategy. The standard approach under REACH is to test for P first, by measurement of the half-life in soil, sediment or water, followed by fish bioaccumulation testing and toxicity testing. This can minimise the need for vertebrate testing. We will present and discuss the following examples of the PBT testing strategy used for suspected PBT substances with 'difficult to test' properties under REACH. For a UVCB containing five constituent groups, a dietary fish bioaccumulation test was used first to assess the B properties of the constituents. For another complex UVCB, degradation testing was performed on a purified fraction to assess its persistency. For an adsorptive substance, a simulation test in water was requested first to minimise the potential for formation of NER by minimising the amount of particulate matter in the test system. For a multi-constituent substance, testing was requested on a specific constituent of the substance which screened to be of most concern using QSAR predictions. **Key words: PBT (persistent, bioaccumulative, toxic), testing strategy, UVCB, REACH**

MO210

Challenges of Testing Chemical Mixtures in Aquatic Toxicology Studies

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A key component of any aquatic toxicology test is the delivery of the test substance in the test system. This is difficult with a single chemical and is even more complicated with mixtures. The more chemicals in the mixture the more difficult it becomes to design a test that maximizes exposure for each chemical. But before one even begins testing, it is important to know 1) how the toxicity of the mixture is to be expressed, 2) physical and chemical properties of the chemical(s), 3) the suitability of a particular test type, and 4) proper analytical methodology. While it is appealing to test at nominal concentrations and not measure each of the components in a mixture, it is also desirable to know which component(s) are causing toxicity or if there is an interaction between chemicals. It is uncertain as to whether the toxicity should be expressed based on the toxicity of the entire mixture, key components, or surrogate measures such as TOC. Most regulatory tests require maintaining concentrations in water between 80 and 120% of nominal for the duration of the test. What does that mean for mixtures? Do all chemicals in the mixture need to be maintained in that range or only the ones suspected to be toxic at concentrations of concern? For a single chemical or mixture it is important to understand the physical and chemical properties of the chemical(s) in order to design a proper test. Prior to testing it is desirable to know if the chemical is volatile, stable in water, its water solubility, whether it adsorbs to components of the test system, or reacts to light. That will determine if the test can be run as a static, static-renewal, or flow-through test. Closed systems can be used for volatile materials, water accommodated fractions were developed for petroleum products and are also useful complex materials with low solubility, saturator columns or passive dosing systems are newer techniques that also are useful. There are other options for bioaccumulation tests in which the test material can be delivered via diet instead of a water exposure or sediment tests in which test materials are added to the sediment for testing. The more chemicals in the mixture the more difficult it becomes to design a test that maximizes exposure for each chemical. Choices and trade-offs based on regulatory concerns will be necessary in designing aquatic toxicology studies for mixtures.

MO211

World distribution of test species for ecotoxicology of antifouling biocides

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Toxicity tests have been used for setting environmental quality standards in many countries and results are important for toxicity evaluation in ecological risk assessments. However, environmental authorities usually require that standard species be used for regulatory purposes. These species distribute mainly in temperate waters, in spite that most of the megadiverse countries in the world are located in the tropics. In order to investigate the representativeness of test species in different continents, this study compiled the world distribution of 92 marine species - of which 15 are standard species for toxicity tests, that have been used to determine the toxicity of 13 antifoulants under lab conditions. GBIF, WoRMS, Algaebase, Molluscabase and Fishbase were used as databases for checking the distribution of each test species, which were categorised by taxonomic groups (microalgae, macrophytes, invertebrates and fish). The occurrence of test species in different continents was analysed compared to the total number of test species. The percent of test species (out of 92) that have been registered for each continent are as follows: Europe and North America 70.7%, Asia 63%, Oceania 45.7%, South America 42.4%, Africa 41.3%, Central America 27.2%, and Antarctica 8.7%. When only standard species are taken into account, the occurrence is reduced in all continents as follows: North America 13%, Europe and Asia 9.8, Oceania 6.5%, South America 8.7%, Africa 7.6%, Central America 2.2%, and Antarctica 0%. Results also show that microalgae test species are usually widely distributed; more than 50% of invertebrate test species are registered for Asia, Europe, North America; and more than 50% of fish test species occur in Europe and North America. In spite of great biodiversity, representativeness of fish and invertebrate test species was low in Central America, South America and Oceania. The results show that the use of native species greatly contributes to increase ecotoxicity datasets and may reduce uncertainties for the establishment of PNECs by increasing representativeness of the ecosystem threatened by antifoulants.

MO212

Investigation of the effect of macronutrients on ecotoxicity evaluation of sewage treatment water to algae

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Whole effluent toxicity test is a method to evaluate ecological effects of samples containing various chemical substances such as industrial wastewater. It is possible to evaluate wastewater without pretreatment.However, that method has a problem that the ecotoxicity of a sample containing a high concentration of nutrient salt such as sewage treated water is masked by effect of nutrient. In this study, the influence of a macronutrients on the ecotoxicity evaluation of sewage treated water using algae was investigated. We conducted toxicity test using the organisation for economic co-operation and development (OECD) TG 201 and short-term test kit including algal cell. In the test-kit including algal cell, 9.6 ml of a medium was dispensed to a culture measuring tube. After that, the frozen algae kit was thawed with a thawing apparatus, and the thawed concentrated algae suspension 400 ?L was added, sealed with a ventilation seal, and cultured for 1 hour in a culture apparatus was used for the test. After completion of the recovery culture, initial value measurement was carried out to ascertain whether recovery culture was normally performed with a high sensitivity luminometer. After the initial value measurement, we dispensed 9.5 mL of the sample to each culture measurement tube, added a total of 18 culture measuring tubes algae suspension by 0.5 ml, sealed with a ventilation seal, a set was incubated in a culture device. After that, the culture was rotated at 24 ± 1 ° C, and the delayed fluorescence after 6 hours and 24 hours was measured. It was conducted the toxicity test at nitrogen concentration 4, 7, 12, 24, 47 mg/L using potassium nitrate. As a result, it was observed growth inhibition at 24 mg/L at test kit. However, it was not observed the increase of growth rate by nitrate, and the masking effect of toxicity was considered to be low. While, it was observed the increase of growth rate by nitrate at TG201. In the test with the addition of dipotassium hydrogenphosphate, Potassium dihydrogenphosphate, or iron fulvate was not confirmed significantly different on both algae growth inhibition test and algae delayed fluorescence inhibition test. In addition to this, the results of evaluating treated water / effluent water at the sewage treatment plant will be reported.

MO213

Interlaboratory comparison test on soil improver quality

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The recycling of nutrients and organic material from waste material helps create a closed nutrient cycle and reduces the need for additional nutrients from non-renewable sources. To increase the market potential for bio-based fertilizer products we need reliable quality assessment tools. Soil improver quality is commonly determined using a test battery of chemical, biological and ecotoxicity tests. An interlaboratory comparison test was organized for determining the quality of two green waste and sewage sludge soil improver samples. The performed analysis were: Germination and root growth of cress, nitrate-N/-ammonia-N ratio, self-heating and CO_2-production. In addition, chemical parameters like dry weight, pH, electrical conductivity, bulk density and organic matter content of samples were measured. These tests are used for determining composition, phytotoxicity, stability and maturity of soil improvers which can be caused for instance by ammonia, ethylene oxide or short chain fatty acids. In total 11 participants took part, of which eight were from Finland. The interlaboratory comparison test was carried out in accordance with international guidelines and the performance of the participants was evaluated by using z scores. Altogether 50 % of the participants used accredited analytical methods at least for a part of the measurands and 100 % of their results were satisfactory. In this proficiency test, 96 % of the results were satisfactory when deviation of 1 pH units and 25–80 % (for other determinations) from the assigned value was accepted. Maturity assessment of these samples, according to criteria from Finnish legislation and results from the laboratories that performed at least three maturity tests, showed that both samples were mature and stable. According to the results, many participants had good practices and manage these analyses well. Other laboratories still need more experience. More detailed guidance on procedures that may affect the results is needed.Further harmonization is recommended e.g. by training courses and updating existing method description to harmonize procedures that affect the results.

MO214

Automatic counting and evaluation in Ecotoxicology assays

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In ecotoxicology assays, reproduction rate, involving the counting of offspring, is often one of the endpoints most frequently evaluated. The counting of organisms has been performed by piking manually every single organism (against a backlight to improve the contrast) in all replicates, which may totalize tens or hundreds of flasks. As stated before, other possible approaches could involve image digital processing but it would involve substantial modifications to present established guidelines, counting extrapolations, and also time consuming. Automatic counting minimizes human error, serializing all the organisms under exposure and providing a chromatic data set for every single organism based on both absorption and scattering light signals. The device forces the passage of all the organisms through a group of optical sensors. The acquired digital signals are then processed in real-time by a computing system in order to automatically count, and to evaluate body length and chromatically characterize each organism. The light absorption from the exposure medium can also be simultaneous registered and it is a relevant feature for ingestion bioassays assessing feeding rates. All the acquired data is stored allowing it to be reprocessed using different detection and characterization criteria and also to be exported in common data processing applications, such as Excel. The device can replace the traditional counting method, hence assuring a greater reliability, making the counts, or its repetition, a much easier and faster process. It can also contribute for diminishing human errors as well as to preserve visual acuity of the technicians, caused by the repetitive use of the traditional counting method. Given the simplicity of the device, this can become an important tool for the automatic counting of organisms, regarding that this are put on a suspension, including evaluating new model species. Concerning the chromatic characterization, this device allows the chromatic characterization of both medium and organisms simultaneously, due to the presence of both receptors and emissaries on top of the collector recipient. This functionality is particularly useful in ecotoxicological studies for the evaluation of the ingestion of the organisms, through determination of the concentration of algae in the medium. The device can also be used in bioassays involving exposure of mixed species hard to differentiate by naked eye.

MO215

Impact of the use of sugar cane vinasse as fertilizer over the years: Terrestrial toxicity bioassay and soil physics study

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Vinasse is the main liquid residue from the processing of sugarcane in ethanol. Over the last 35 years the practice of fertigation of vinasse in sugarcane crops has been carried out as a way of discarding this residue. This study aimed to evaluate the impact that vinasse can cause in areas that receive it for approximately 5, 15 and 30 years, using Rinochricus padbergi as a bioindicator and soil physics tests to determine the mobility of potassium and nitrate ions. The soil samples were collected in sugarcane crops that received vinasse for 5, 15 and 30 years and two control areas (S5, S15, S30, SC and SS, respectively). The bioassay was performed in triplicate, each terrarium containing 7.5 kg of soil and 20 animals with exposure for 21 and 42 days. After exposure, three individuals from each terrarium were dissected for midgut collection for histopathological analysis and labeling of HSP70 heat shock protein. In order to verify the mobility of the ions in the soils, the vinasse was percolated in segmented columns. In the terrestrial toxicity bioassay there was a high mortality of the diplopods exposed to the soil S30 in 21 days; after 42 days of exposure all individuals exposed to the soil S30 died and only four survived in the soil S15. Midgut histological analyzes revealed a significant thickening of epithelial cells brush border, as well as loss of adhesion and presence of cytoplasmic vacuoles in epithelial cells in some animals. There was also an increase in spherocrystals in hepatic cells. From analysis of the HSP70 labeling, there were statistically significant differences in individuals exposed for 21 days in soil S5 and S15 and after 42 days in soil S5 when compared to controls. In the soil physics tests it was observed that potassium presented low mobility in soils, due to its high adsorption, being present in higher concentrations in the upper rings of the columns. For nitrate, the highest concentrations were found in the lower rings, since it is an anion being easily carried along the column. Considering the results of soil physics studies, it is noted that the prolonged use of fertirrigation in soils may be related to the mobility of the compounds and the adsorption of potentially toxic compounds. The results obtained through terrestrial toxicity bioassay suggest toxic action of the soils that received vinasse as fertilizer, being verified significant effects even in soil that received vinasse for only 5 years.

MO216

Sugarcane vinasse treated for neutral pH adjustment reveals low toxicity in millipedes

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One of the main final destinations of sugarcane vinasse has been fertirrigation. Legislation for its adequate use has been developed in order to respect the characteristics and limits of the soil to receive this residue. Its use as fertilizer shows numerous benefits, however several researchers have revealed that vinasse causes damage to representatives of soil edafic fauna. As one of the major problems presented by vinasse is its acidic pH, this study proposed to treat vinasse with lime (CaO) in order to adjust its pH to 7,0 (neutral) in an attempt to reduce its toxicity for later use in crops. For this purpose, millipedes of the species *Rhinocricus padbergi,* well established animals for soil ecotoxicological tests, were used as biondicators organisms. In order to analyze the toxicity of treated vinasse, morphological alterations in the cells of millipede midgut were observed. The animals (n=30 per treatment) were exposed to five treatment groups: a control group (animals exposed to soil from the site where the animals were collected); a group of animals exposed to vinasse in its raw form, prior the treatment with lime, in the concentration established by the Brazilian legislation; a group exposed at double concentration allowed by the legislation, simulating a situation of super dosage; a group exposed to treated vinasse with lime (pH adjusted to 7,0) in the concentration established by the legislation; a group exposed to treated vinasse at double concentration allowed by the legislation. The animals were exposed for 30 days. After this period, millipedes were dissected for removal of the midgut, which was processed for analysis in Transmission Electron Microscopy (TEM). The animals exposed to the control group presented the midgut with morphological integrity, as expected. The animals exposed to the raw vinasse, at both concentrations, presented several morphological changes in the cells of the midgut, as nuclear alteration, reduced microvilli and loss of plasma membrane integrity. The animals exposed to the treated vinasse, at both concentrations, presented less cellular damages when compared to the animals exposed to the raw vinasse. According to the results, the treatment of the vinasse with lime is a viable alternative to reduce its toxicity.

MO217

Experimental membrane-water partition coefficients are becoming available to aid ionogenic surfactant risk assessment

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Surfactants are important ingredients in various household products, personal care products and many different industrial processes. They are often used and applied in relatively high concentrations on a daily basis, which leads to relatively high concentrations in waste water streams, of which resilient fractions may drive a constant emission into aqueous environments receiving (treated) waste water. A first aim of this study was to provide an overview of surfactant substances registered in REACH. Over 150 substances were retrieved, covering different types of polar headgroups (e.g. sulfonates, sulfates, amines, ethanolamines, diamines, amine-oxides, quaternary ammonium compounds) and different hydrophobic tails (e.g. petroleum origins, naphthalene structures, tallow based origins). Many registered surfactants are complex technical mixtures of ionogenic head groups and linear or branched hydrophobic alkyl chain lengths ranging between C_{12}-$C_{>18}$. \n As for many ionogenic compounds, the environmental fate assessment of ionogenic surfactants is complicated because it is not clear how to parameterize critical partition coefficients such as K_{ow}, K_{oc}, or how to model uptake in organismal tissue. Various techniques to derive logP for surfactants are described in these REACH dossiers, with conflicting argumentations on which is most relevant. It is well known that K_{ow} is a problematic parameter for surfactants, and that only experimental soil-water partition studies or bioconcentration studies provide adequate insight. To avoid and/or reduce costly and unethical numbers of animal tests, insight in the key parameters of pure surfactant components driving uptake in biota is highly needed. The phospholipid-water partition coefficient (K_{MW}) is considered to be the dominant contributor to the overall tissue-water partition coefficient for ionogenic surfactants, because membranes lipids allow for both ionic interactions at the zwitterionic head groups and hydrophobic interactions at the membrane core. Therefore, the second aim of this study was to provide an overview of 56 surfactant substances for which K_{MW} values have been reported, as an already available, consistent and meaningful partition data set for surfactants. The third aim of this study was to provide an overview of the preliminary K_{MW} data set generated for 40 additional surfactants during the CEFIC ECO37 project.

MO218

Effects of produced water components on early life stages of cold-water marine fish

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Regular discharges of produced water from the offshore oil and gas industry represents the largest direct discharge of effluent into the marine environment worldwide. Produced water contains an aqueous mix of formation water, oil and/or gas from the reservoir, injected freshwater or brine water and added production chemicals. Organic compound classes typically reported in produced water include saturated hydrocarbons, monoaromatic and polyaromatic hydrocarbons (MAHs, PAHs) as well as oxygenated compounds, such as phenols, acids and ketones. Total (for the year 2016) produced water releases from activities on the Norwegian continental shelf alone was estimated to include 1 600 tons of crude oil, 2221 tons BTEX (benzene, toluene, ethylbenzene and xylenes), 576 tons phenols, 28 438 tons organic acids and 126 tons PAHs. This forms a cocktail of known and suspect compounds causing acute adverse effects, but limited knowledge is yet available on the sub-lethal toxicity of produced water to marine species. In the present work, we conducted a 4-day exposure of embryos of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) to produced water extracts equivalent to 1:50, 1:500 and 1:5000 times dilutions of raw produced water in natural sea water. During exposure, embryonic heart rate was significantly reduced for both species at the highest exposure concentrations. After exposure, eggs were transferred to and kept in clean sea water and kept until 3 days post hatch. No significant reduction in survival or hatching success was observed. However, the exposed cod eggs hatched earlier in a concentration-dependent manner. After hatch, larvae exposed to produced water extracts were smaller. Furthermore, the larvae displayed signs of cardiotoxicity, jaw and craniofacial deformations in a concentration-dependent manner. In a follow-up study, we extracted and fractionated produced water into 12 fractions with different component groups. Experiments are currently on-going to identify and determine which specific component groups are the main drivers for the observed fish toxicity.

MO219

Optimising the feeding of the Mayfly Cloeon dipterum under laboratory conditions and the influence of diet on growth and emergence

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The pre-adult stages of mayflies (Ephemeroptera) reside in freshwater habitats such as ponds, streams, rivers and lakes. Recent studies have found that, in some instances, mayfly larvae can be more sensitive to Plant Protection Products (PPPs) than other benthic macroinvertebrates. One common issue with the use of mayfly larvae in chronic toxicity testing has been the optimisation of larval diet to ensure successful growth through to emergence. Attempts have been made to develop a standardised toxicity test method for non-European species such as *Neocloeon triangulifer* (US) and *Hexagenia limbata* (US, Canada); however, little progress has been made using European species. The mayfly species, *Cloeon dipterum* is highly abundant throughout the British Isles and across Europe. In their natural environment they would feed upon available detritus and algae. Using wild-caught mayfly to maintain a culture in a laboratory environment can be problematic, in terms of what diet to feed them and also being able to culture algae or diatoms for feeding purposes. In this poster we will present our development work identifying different food types that *Cloeon dipterum* larvae can be fed, including algae (*Desmodesmus*), crushed alder leaves, and vegetarian fish food, and describe what impact the different diets have on development, growth and emergence rate.

MO220

Non-standard biotests - Intelligent testing to combine different needs and requirements for experimental and modelling approaches

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In recent years biotests differing from standard procedures become more and more important for the environmental risk assessment of plant protection products. For higher-tier testing test designs are adapted to frequently complex issues in order to contribute to a more realistic risk assessment if applied for instance in SSDs or in the context of effect modelling. In addition to variations in exposure (e.g. peak scenarios) non-standard species as e.g. mayfly, stonefly and caddisfly larvae, isopods or gammarids are increasingly being tested. Historically the design of experiments used for classical higher tier risk assessment has other requirements than experiments used for modelling purposes (e.g. number of observation times, number of replicates). However, to minimize the testing effort in this context it is of particular importance to define certain basic test requirements to ensure the best possible use of the generated data for other issues (intelligent experimental design). Thus, we aim to use standard experimental data for the parameterization or validation of TKTD effect models and vice versa. In addition, it would be useful to design the test conditions in such a way that the experimental results can also be used for mechanistic population modelling. To ensure multi-usable and comparable test results, the selection of the test species and their origin, the developmental stage of the test species, the toxicological endpoints (lethal and sub-lethal), the number of replicates and the number of observations as well as the test duration must be clearly defined. Furthermore, adapting the test conditions to the ecological requirements of the specific test species (e.g. in terms of the selection and characterization of food, selection of test temperature) minimizes the laboratory stress for the organisms, and thus results in increased validity, and allows an easier integration into more advanced model applications. We will present basic requirements for biotests with non-standard species and discuss the data needs in particular for the context of different effect modelling approaches.

MO221

Occurrence and environmental risks of pharmaceuticals and personal care products in Guanting Reservoir and its upstream rivers

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Abstract: Eighteen selected pharmaceuticals and personal care products (PPCPs), consisting of five non-antibiotic pharmaceuticals (N-APs), four sulfonamides (SAs), four tetracyclines (TCs), four macrolides (MCs), and one quinolone (QN) were detected in surface water and sediments from Guanting Reservoir (GTR) and its upstream rivers in north China. Acetaminophen, caffeine, chlorotetracycline, and ofloxacin were detected with 100% frequency in the surface water of GTR and its upstream rivers, while diltiazem was also detected with 100% frequency in surface water from the reservoir's upstream rivers. Acetaminophen and caffeine were detected with 100% frequency in sediments from GTR and its upstream rivers, while high concentrations of ofloxacin in GTR, and carbamazepine, tetracycline, and chlortetracycline in upstream rivers were also detected in 100% of samples. Five N-Aps, especially acetaminophen and caffeine, were prominent pollutants. The mean concentrations of acetaminophen were 155 and 302 ng•L^{-1} in surface water and 529 and 202 ng•g^{-1} in sediments from GTR and upstream rivers, respectively. The mean concentrations of caffeine were 208 and 338 ng•L^{-1} in surface water samples and 1430 and 1020 ng•g^{-1} in sediments from GTR and upstream rivers, respectively. The geographical differences in PPCP concentrations were largely due to anthropogenic activities. Sewage discharged from Zhangjiakou City and human activities around the GTR basin were the main sources of PPCPs in this area. An environmental risk assessment for the worst-case scenario was undertaken using calculated risk quotients, which indicated a medium risk from erythromycin in GTR and a high risk in its upstream rivers.\nKey words: Occurrence, environmental risks, pharmaceuticals and personal care products (PPCPs), Guanting Reservoir (GTR)

MO222

TOXICITY TESTING OF WASTEWATER WITH GREEN ALGAE RAPHIDOCELIS SUBCAPITATA (=PSEUDOKIRCHNERIELLA SUBCAPITATA)

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In accordance with the provisions of the Slovenian General Emission Regulation, it is necessary to determine concentrations of selected chemical parameters as well as the toxicity for the aquatic organism Daphnia magna (small planktonic crustacean, water flea) in industrial wastewater. In the case where herbicides are present in wastewater, it would be more appropriate to test the toxicity of organisms from first trophic level such as green algae for a more realistic environmental risk assessment. In this research we discussed the effect of various wastewaters and herbicide terbutylazin on the aquatic ecosystem. Our intention was to investigate and justify the introduction of the toxicity test with the green alga Raphidocelis subcapitata into Slovenian legislation.

Raphidocelis subcapitata was formerly known as Pseudokirchneriella subcapitata or Selenastrum capricornutum. Aquatic toxicity test with water flea was carried out in accordance with the standard method OECD TG 202, and the freshwater algae growth inhibition test was carried out using the standard method OECD TG 201. We have determined that wastewaters have a different effect on the water flea mobility and on the growth of green algae. Herbicide terbutilazin, even at the highest concentrations used, did not exhibit toxic effects on daphnids, whereas in the case of algae concentrations were inhibitory at all levels used in tests.

MO223

Harmful substances from reclaimed asphalt and their effect on living organisms

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The composition of reclaimed asphalt (RA) is an important issue due to its further use for renovations or construction of new roads. All these materials must meet the conditions required for environmental protection. The occurrence of pollutants in RA is related to the actual composition of the original material (e.g. the presence of tar in the binder) and the formation of pollutants during road traffic (exhaust gases, abrasion of the tire and brake lining). The paper discusses an experiment whose aim was to assess the leaching of harmful substances from the RA repository under real conditions and comparing the acquired data with the results of the leaching tests of these materials in laboratory conditions. The experiment consisted of determining the contents of polycyclic aromatic hydrocarbons (PAHs) and metals in four types of samples - the RA material, laboratory-prepared water leachate from this material, seeping water obtained from the repository under real conditions and rainfall from the repository site. Chemical analyses of these samples were supplemented by ecotoxicological tests that characterize the effect of the material on living organisms. Seeping water was found not to have significant negative effects on living organisms, although it was relatively polluted by selected compounds (metals and PAHs). Laboratory prepared water leachate of the material have revealed the greater negative effect on living organisms than the seeping water, although the concentrations of analyzed substances were much lower there. Thus, the leachate could contain "unknown" substances from the leaching process in the laboratory that were not analyzed and could occur in high concentrations and are responsible for toxic effects on living organisms. The presented experiment shows the benefits of ecotoxicological tests for evaluation of hazardousness of RA materials for freshwater ecosystems. Using only chemical analysis in assessing the possible harmful effects of RA materials could provide misleading information. The results of the experiment further indicate the importance of determining the pollutants in the water leachate of a material rather than just determining the content of harmful substances in the dry matter. The reason is the contact of the material with rain or underground water under real conditions, in which foreign substances can be released into the environment.

MO224

Time-course of coiling activity in zebrafish (Danio rerio) as an endpoint for developmental neurotoxicity - hidden potential and underestimated challenges

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First locomotor activity in zebrafish embryos is characterized as spontaneous one-sided tail coils that originate from a single neural circuit in the spinal cord. According to literature, this coiling activity is driven by periodically depolarizing spinal neurons independent of light or touch stimuli. Throughout the embryonic development until late pharyngula stage, coiling movements become more complex (e.g., modifiable by external stimuli), and multiple neural circuits are integrated. Early tail coiling in zebrafish has been subject to numerous studies assessing developmental neurotoxicity (DNT). Many of these have been criticized for bearing considerable limitations in terms of methodology and result interpretation, and only a handful of studies have examined coiling activity with regard to its actual suitability as DNT endpoint. Therefore, effects of five neurotoxic compounds (ethanol, cadmium, dichlorvos, fluoxetine, and citalopram) on coiling activity of zebrafish embryos until the late pharyngula stage (47 hpf) were investigated. Coiling activity in zebrafish was analyzed on an hourly basis under infrared illumination with a common day/night light cycle (14/10 h). Substantial new insights were gained concerning the development of first locomotor activity in the zebrafish embryo. Results were evaluated against the background of substance-specific embryo toxicity (fish embryo toxicity test, OECD 236). Findings revealed distinct effects on coiling activity by the test compounds, which differed with respect to their neurotoxic mode of action. Effects on coiling activity could not be interpreted separately from substance-specific embryo toxicity. To the best of our knowledge, the present study is the first of its kind investigating the development of coiling activity in zebrafish embryos by means of non-visual light. On the basis of an enhanced test protocol, various challenges could be identified which may arise during the examination of coiling activity as an endpoint for DNT in zebrafish embryos. On the other hand, results highlight the potential of an improved coiling assay to distinguish between chemicals with different neurotoxic modes of action. The present work is part of the project “Effect Network in Water Research” and has been funded within the framework of „Wasserforschung Baden-Wuerttemberg".

MO225

Effects of CaCO3 mine tailings on the marine copepod Calanus finmarchicus

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The rapidly increasing demand for mineral resources is driving mining activities worldwide. Mining generates extensive quantities of waste that needs to be disposed of. In Norway, mine tailings are disposed directly in fjords in several locations. During marine mine tailing disposal large quantities of fine inorganic particles, associated metals and processing chemicals can be introduced into the marine environment. Exposure to mine tailings, especially to inorganic particles, does not necessarily cause acute toxicity and mortality, but can cause physiological stress and reduced food intake impacting organisms' energy budget. In this study we investigate the effects of tailings deriving from a $CaCO_3$ processing plant on different life stages of the marine filter feeding copepod *Calanus finmarchicus*: eggs to nauplii stage 3 (N3), N3 to N5/N6, and adult individuals. The tested tailings contain a large fraction of very fine particles as well as floatation and flocculation chemicals. The tailing stock dispersions were prepared as 1g/L wet weight (30% dry mass) in seawater, were left for settling of larger particles for 20 min and subsequently decanted. Decanted dispersions were used as high (H) exposure concentration (approximately 2 million particles/mL), or diluted to middle (M; 1:1) and low (L; 1:10) exposure concentrations. Exposure (96 h) to $CaCO_3$ tailings did not significantly reduce hatching success and survival up to N3 life stages. However, when deprived of food, all tailing exposures caused an almost 100 % mortality in the first feeding stage nauplii (N3), with tailings found in the guts of dead nauplii. Such mortalities were not observed in nauplii (N3) and N5/6 that were additionally provided with algae during tailing exposure, even though uptake of tailings together with algae occurred in 70 – 96 % of the exposed individuals. Similarly, short term exposure (72 h) did not lead to acute mortality in adult copepods, however, 86±11% of the exposed individuals had taken up tailings into their guts. Impacts of tailings on oxygen consumption, developmental rates, size and activity levels are currently ongoing. All obtained data will be integrated into energy budget models in order quantitatively test the hypotheses that mine tailing particles can exert sublethal effects through reduced energy intake versus increased costs for maintenance.

Antimicrobial Resistance in the Environment (P)

MO226

Environmental issues due to antibiotic occurrence in manure and digestate: the AZeRO antibiotics Project

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Antibiotics are emerging contaminants with two-fold main impacts: they can affect some microbial populations interfering with biogeochemical cycles and can act as micropollutants active in the transmission of resistance genes. For these reasons, antimicrobial resistance has become an emerging threat for human, animal and ecosystem health. Huge amounts of the produced antibiotics are used in livestock farms and the persistence of antibiotics in the cattle manure can be cause of environmental contamination considering that it is often spread on the land as a fertilizer. Moreover, owing to the practice of energetic biomass wastes valorization, manure is increasingly used to feed biogas plants. The presence of antibiotics into the digesters can affect the performance of the anaerobic digestion process leading to sub-optimal biogas productions, and in the case of their persistence, a compost of low quality is obtained. The AZeRO antibiotics Project (*AZeRO Antibiotici Prot. N.ro 85-2017-15065" – LAZIO INNOVA - Progetti di gruppi di ricerca*) relies on a joint collaboration between the Italian Ecology lab of IRSA-CNR and the Biomass and Biotechnologies for Energy Lab of ENEA. It aims at: assessing the presence of antibiotics in manure and digestate at a regional scale (Lazio, Italy) considering farms that own biogas plants; studying the kinetics of antibiotic degradation, in the anaerobic digestion process (anaerobic condition) and in the storage and spread phases of digestate (aerobic condition). Preliminary studies showed that the genes of resistance can be transferred from digestate bacteria to microbial communities of agricultural soil. The three-year Project also aims to find green solutions to manage antibiotics and resistance genes spreaded in the environment. Key words: Antibiotic resistance, manure, anaerobic digestion, digestate.

MO227

Methods for the risk assessment of antibiotic resistance and related genes in the environment

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The origins of antibiotic resistance in the environment is relevant to human health because of the increasing importance of zoonotic diseases as well as the need for predicting emerging resistant pathogens. Antibiotics are used in diverse settings for food production. Domestic animals are treated with antibiotics for both curing disease and promoting growth. Moreover, aquaculture relies on antibiotics to manage infectious disease. Wastewater treatment plants receive sewage from various sources, including hospitals and households which are both important sources of antibiotics and their residues, and antibiotic resistant bacteria. Risk assessment of antibiotic resistance is complex. It should include at least quantitative information of the gene, sequence of the gene, host cell of the gene and genetic environment of the gene (e.g. presence in mobile DNA element). We have used the combination of different methods for obtaining that information: Parallel quantitative PCR array for high throughput quantification (1), epicPCR(2) for host information and Inverse-PCR(3) for analysis of the genetic environment. Inverse-PCR and epicPCR combined with DNA sequencing resolve also the sequence of the resistance gene. Our results demonstrate that human activities results to the increase to the abundance of antibiotic resistance genes. In many cases the genes are located in mobile genetic elements with increases the probability of transfer of the them between bacterial species. The host range information obtained by epicPCR revealed wide diversity on the host range of the antibiotic reistance genes in different environments. Our results can be used for the development ecotoxicological risk analysis for antibiotic resistance. (1) Karkman, A., Johnson, T.A., Lyra, C., Stedtfeld, R.D., Tamminen, M., Tiedje, J.M. and Virta, M. (2016) FEMS Microbial Ecology 92 (3): fiw014 (2) Spencer, SJ., Tamminen, MV., Preheim, SP., Guo, MT., Briggs, AW., Brito, IL., Weitz, DA., Pitkänen, LK., Vigneault, F., Virta, MP. and Alm, EJ. (2016) ISME Journal 10:427-436 (3) Pärnänen, K., Karkman, A., Tamminen, M., Lyra, C., Paulin, L., Hultman J. and Virta, M. (2016) Scientific Reports 6: 35790

MO228

Detection of low levels of antibiotic resistance genes in aquatic environments

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Detection of low levels of antibiotic resistance genes in aquatic environments
Aminur Rahman†, Faisal Ahmad Khan†, Jana Jass † Contributed equally The Life Science Center - Biology, School of Science and Technology, Örebro University, SE-701 82 Örebro, Sweden Abstract Low levels of antibiotics and pharmaceuticals are regularly released into natural aquatic environments via wastewater, thus there is a concern that these anthropogenic practices may serve to create hotspots for antibiotic resistant bacteria. Although it is important to study antibiotic resistant bacteria, antibiotic resistance genes (ARGs) associated with clinically relevant human pathogens are considered an emerging environmental contaminant. There are several methods to detect ARGs in the environment, including, metagenomic analysis and various PCR methods. However, it is difficult to detect very low levels of ARGs with these methods from environmental samples. The aim of this study was to develop a sensitive and efficient method to detect ARGs of culturable coliforms and Gram-negative bacteria in aquatic environments where there are low levels of ARG. Water samples were collected from a river up-stream and down-stream of a wastewater treatment plant in Örebro, Sweden. The water was filtered using 0.45 μm cellulose filter and incubated overnight at 37ºC on coliform selective chromocult agar to enrich the growth of Enterobacteriaceae. A comparison was made between DNA isolated directly form the waters and from enriched bacteria growing on chromocult agar for the presence of 84 ARGs using DNA qPCR arrays. Different classes of beta-lactamase, fluoroquinolone, tetracycline, aminoglycoside resistance genes and multidrug resistance efflux pump genes were detected in both enriched and non-enriched samples. However, there was a significantly higher number of ARGs detected in enriched samples than that observed in non-enriched samples. The results indicate that the enrichment of Enterobacteriaceae and Gram-negative bacteria can provide a more accurate indication of the distribution of environmental contamination by ARGs. **Key words:** Enterobacteriaceae, Coliform bacteria, Antibiotic resistant bacteria, DNA qPCR array

MO229

Screening for a range of antibiotic resistance genes in the Swedish surface water environment

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The presence of antibiotic resistance genes (ARGs) in aquatic environments poses a threat to human and environmental health. Water bodies which receive effluent discharge of wastewater treatment plants (WWTPs) are an important media to evaluate ARGs entering the environment. A few European studies have reported ARGs in recipient waters, but such data remains very limited in Sweden. The key objective of this study was to assess the prevalence of ARGs in the Swedish surface water environments. Specially, it aimed to (a) collect recipient water samples, downstream of the municipal wastewater treatment plants, as well as upstream water samples where possible, and (b) screen for a wide range of ARGs in the target water environment. Various ARGs and other genes in the samples were identified and quantified using a high-throughput quantitative polymerase chain reaction (qPCR) method with 384 primer sets. A total of 152 ARGs were detected in all the samples, conferring resistance to aminoglycoside, amphenicol, β-lactam, genes classified as multidrug resistance, macrolide lincosamide streptogramin B, sulfonamide, tetracycline, trimethoprim and vancomycin. Additionally, integrase genes of class 1, 2 and 3 integrons as well as six other resistance genes were found in the samples. More genes were detected in the downstream than the upstream samples, particularly for the Uppsala's County site. Our results showed that antibiotic resistance was ubiquitous in the studied water bodies and WWTP effluents were an important impact source for ARGs. Since the recipient water eventually connects to the largest drinking water reservoir in Sweden, there is a potential risk of spreading antibiotic resistance genes to the source of drinking water. Overall, our finding provided a new insight into the occurrence and abundance for a wide range of ARGs and other genes in the Swedish aquatic environment. This could assist to select specific sites for assessing temporal changes of ARGs in such a context of Sweden in the future. **Keywords:** river water, antimicrobial resistance, antibiotic resistant bacteria, qPCR **Presentation preference:** platform presentation

MO230

Presence and fate of antibiotics and antibiotic resistant genes in Finnish wastewaters

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The constant release of antibiotics (even in very small concentrations of nanograms and micrograms per litre) in natural waters not only cause toxic effect on aquatic organisms but also spread antibiotic resistant genes (ARGs) to the environment threatening the future therapeutic efficiency of existing antimicrobial drugs. Urban wastewater treatment plants (WWTPs) are among the main sources for the release of antibiotic resistance into the aquatic environment. At the same time, the data on the removal of antibiotic resistant genes (ARG) are very limited and there is a big gap of knowledge on dynamics of antibiotic resistant bacteria (ARBs) and ARGs during wastewater treatment process. In this study, wastewaters of seven Finnish WWTPs are studied in order to gain the total profile of the antibiotics and antibiotic resistance genes (ARG) in influent and effluent wastewaters.The target antibiotics are analyzed by ultra-performance liquid chromatography mass spectrometry. The presence of ARGs is studied by *SmartChip* Real-Time PCR System. The data on antibiotics usage, persistence in wastewaters, removal efficiency are compared with the spread of ARGs in treated wastewaters. The results demonstrate the collective data on presence and fate of 49 antibiotics and 216 ARGs in Finnish wastewaters. This knowledge can be used for the assessment of the commonly used antibiotics environmental safety.

MO231

Field-compatible protocol for detecting bioavailable antibiotic contamination without pipetting steps

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The discovery of antibiotics has had a crucial influence on our civilization, but their efficiency is compromised by accelerating evolution of antibiotic resistance that seems to be a consequence of rampant use of antibiotics. Antibiotics are used in treatment of infections in humans and animals, but also as growth promoters in livestock production and against plant pathogens in crop production. As a result of the high use in agriculture and in humans as well as wastewater discharges from pharmaceutical factories, antibiotic contamination has been found from various foodstuffs, soils, sediments and watercourses. The role environmental of antibiotic contamination in the emergence of antibiotic resistance is yet to be defined and an important question is if the antibiotic residues in the environment can select resistance in bacterial communities, i.e. if the contaminants are bioavailable for bacteria carrying resistance genes. Whole-cell bioreporters are living organisms that produce measurable signals in the presence of target chemicals. Thus, using bioreporters for detecting environmental contaminants would reflect biological effects of these pollutants. However, bioreporters are not widely used in field studies. One possible explanation for this is that many of the bioreporter field protocols are suitable only for liquid samples or include pipetting steps, which is a demanding task outside laboratory. We present a bioreporter protocol for detecting tetracycline contamination without pipetting steps or sample type requirements. The protocol utilizes polyester swabs that are commonly used in cleanroom technology. For testing the applicability of the protocol, we generated test samples in Milli-Q water, milk and soil matrixes with known tetracycline concentrations up to the maximum tetracycline residue limit of milk set by the EU regulation. The swabs were first dipped to the bioreporter cell cultures and then to test samples, following incubation and luminescence measurements. The standard deviation of measurements from ten replicate swabs ranged 4-19 %, which can be expected also with pipetting protocols. The test samples with lowest tetracycline concentration (5 ng ml^{-1}) could be distinguished from the control samples (0 ng ml^{-1} tetracycline). Our results show that swabs can be used together with luminescent whole cell bioreporters, enabling rapid screening for contaminated foodstuffs and making it possible to conduct the measurements in field conditions

MO232

Study of Antimicrobial Resistance in pristine area

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Nowadays the antimicrobial resistance (AMR) is a European and global concern which has been highlighted as a catastrophic threat for human health. Several studies had pointed out that the aquatic environments may play an important role in the AMR acting as a reservoir and spreading the resistant bacteria and antimicrobial resistant genes (ARG). Although it has been shown the presence of resistant bacteria and ARG in highly anthropogenic areas such as the effluents from the waste-water treatment plants, the AMR is also a natural phenomenon, which is not fully investigated. The aim of the current study is to characterise the natural background of the AMR in a pristine aquatic environment and in microcosm studies to assess the minimal antibiotics' concentration that might induce resistance. In this study, water samples were collected from an alpine river, Rio Variola in Monte Ossolano (Domodossola, Italy) during July, September and October in 2018. To investigate the natural background, a small water volume was plated on agar plates in presence of antibiotics and without antibiotics as a control. The other volume was used to perform microcosm studies. The microcosms were exposed to low concentrations (0-100ng/L) of three antibiotics: erythromycin, amoxicillin, and tetracycline. For the background study, the water from Rio Variola, showed bacteria resistant to ampicillin, amoxicillin, kanamycin, and erythromycin in July and November and only to erythromycin and kanamycin in September. For the microcosm study, after 7 days of exposure, it was observed changes in the occurrence of antimicrobial resistant bacteria on agar plates. Cell number also showed differences after 11 and 13 days of exposure to erythromycin and tetracycline at concentrations of 50ng/L and 100ng/L respectively. This change on the microcosm could explain the variations of bacterial resistance during the experiment. This study demonstrates that resistance to antibiotics occurs naturally in the environment. Furthermore, the low concentrations of antibiotics can have an effect on the bacteria communities and may help in the maintenance of antimicrobial resistance in the environment. Further experiments using the Next Generation Sequencing will allow to characterise the bacterial community changes and to identify the resistance genes.

MO233

Selective potential of sulfamethoxazole in natural microbial communities

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Antibiotic large-scale production and use in human and veterinary medicine, agriculture and aquaculture practices result in a flow of antibiotics, antibiotic-resistant organisms and antibiotic-resistance genes into the natural environment. Recent studies using single species assays showed that selection for antibiotic resistance occurs at very low antibiotic concentrations, suggesting a correlation between the environmental antibiotic concentrations and the spread of antibiotic resistance. Currently, there are few data about selection for and mobilisation of resistance genes in complex natural microbial communities representing real conditions. Sulfamethoxazole (SMX) is a frequently prescribed and consumed antibiotic worldwide, leading to its ubiquitous presence in the natural environment. Thus, the evaluation of the relationship between SMX concentration and the selection for antibiotic resistance in a real community context is necessary for environmental risk assessment. The aim of this study was to investigate the selective potential of SMX in natural systems containing natural microbial communities from different environmental compartments. Initially, different week-long evolution experiments were performed by exposing the microbial community from municipal wastewater to different SMX concentrations (2, 20, 200, 2000 and 20000 µg/L), in order to determine the minimal selective concentration (MSC) of SMX that caused positive selection for resistance. The selection coefficients were calculated based on changes in the class 1 integrase (*intI1)* gene prevalence over time. Results showed that the selective effect of SMX occurred when the antibiotic concentration was between 2000 and 20000 µg/L. However, it is worth to highlight that this range value was higher than that usually found in natural environments. In addition, different SMX biodegradation experiments were conducted in microcosm studies by adding SMX to natural river water (500 µg/L SMX) or to agricultural amended soil (20 mg/kg) both containing the indigenous microbial communities. In these experiments, the correlation between the change in *intI1* prevalence and SMX degradation was evaluated. The results confirmed the role of the SMX in selection for resistant microorganisms at the study concentrations.

MO234

Modelling environmental antibiotic-resistance gene abundance: a meta-analysis

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The successful treatment of infectious diseases heavily relies on the therapeutic usage of antibiotics. However, the overreliance on the use of antibiotics in humans and animals leads to increasing pressure on bacterial populations in favour of resistant phenotypes. Antibiotics reach the environment from a variety of emission sources and are being detected at relatively low concentrations. Given the possibility of selective pressure to occur at sub-inhibitory concentrations, the ecological impact of environmental antibiotic levels on microbial communities and resistance levels is vastly unknown. Quantification of antibiotic-resistance genes (ARG) and of antibiotic concentrations is becoming commonplace. Yet, these two parameters are often assessed separately and in a specific spatiotemporal context, thus missing the opportunity to investigate how antibiotics and ARGs relate. Furthermore, antibiotic (multi)resistance has been receiving ever growing attention from researchers, policy-makers, businesses and civil society. Our aim was to collect the limited data on antibiotic concentrations and ARG abundance currently available to explore if a relationship could be defined in surface waters, sediments and wastewaters. A metric of antibiotic selective pressure, as measured by the concentration and effect of antibiotics, was used to correlate the presence of antibiotics in the environment to total relative abundance of ARG while controlling for basic sources of non-independent variability, such as country, year, study, sample and antibiotic class. The results of this meta-analysis show a significant effect of antibiotic pressure and type of environmental compartment on the increase of ARG abundance even at very low levels. If global environmental antibiotic pollution continues, ARG abundance is expected to continue as well. Moreover, our analysis emphasizes the importance of integrating existing information particularly when attempting to describe complex relationships with limited mechanistic understanding.

MO235

Improved marine environmental risk assessment for pharmaceuticals in marine aquaculture

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Pharmaceuticals are detected in waterbodies all over the world. Antibiotics are of special concern for the environment (e.g. specific activity against prokaryotes, possible formation of microbial resistance). For the approval of pharmaceuticals in marine aquaculture, no marine prokaryotic test system is standardised and implemented in the risk assessment. Moreover, the question, if marine cyanobacteria can act as transfer factor for antimicrobial resistance is so far neglected in research. The akute marine sediment test with the amphipod *Corophium volutator* according to ISO EN DIN (16712) is applied in the European environmental risk assessment, but a more holistic approach considering effect on its microbiom and sublethal parameter are missing. Marine cyanobacteria are of high importance for the nitrogen cycle and primary production especially also in respect of climate change. In order to assess the effects of antibiotics to marine cyanobacteria, a marine cyanobacteria growth inhibition test (72h) with the species *Anabaena spec.* was developed. To investigate the potential of antibiotic resistance in marine cyanobacteria a multi generationtest with repeated exposition was developed. These results were compared to results from a standadized marine eukaryotic algae test. Antibiotics applied in aquaculture reach directly or indirectly not only the waterphase but also sediments. For the environmental risk assessment of single substances and sediments the acute amphipod test with *Corophium volutator* (EN ISO 16712) is an important test system. But the parameter mortality seems not reflect the effects to the species sufficiently, sublethal effects and effects of on the microbiom are of environmental importance and thus investigated in this study. Due to its environmental relevance, seven antibiotics were selected for both test systems. Environmental concentrations of antibiotics ($\mu g\ L^{-1}$) have an impact on ecosystem relevant non-target organisms. Marine cyanobacteria are more sensitive than marine eukaryotic algae to antibiotics in bioassays. The LC_{50} value for *C. volutator* and antibiotics is not sensitive enough to reflect the effects of antibiotics to the sediment dwelling amphipod Sublethal parameters are more suitable for an environmental risk assessment of antibiotics in the marine (sediment) environment. A underestimation of ecotoxicological risk in both cases is possible.

MO236

Antibiotics and antibiotic resistances in the environment - Options for action

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Large quantities of antibiotics are used in both human and veterinary medicine [1, 2]. Once partially metabolised and excreted by humans or animals, antibiotics are transported into the environment via wastewater, the spreading of sewage sludge or manure. Antibiotics can also enter ground or drinking water from surface waters and soils. In Germany, antibiotics have been regularly detected in all environmental compartments in recent years. In this context, antibiotics can inhibit the growth of cyano- and soil bacteria as well as plants and thus damage both aquatic and terrestrial ecosystems. Studies demonstrated that environmental bacteria are resistant to a variety of antibiotics and that this reservoir of antimicrobial resistances (AMR) is steadily increasing in the environment. In fact, there is evidence that at least some clinically relevant resistance genes from bacterial species originate from the environment. Manure and their digestates, for example, are often contaminated with antibiotic-resistant bacteria (ARB) and constitute as a "hotspot" for the transmission of resistance genes. In particular, the combination of various factors, such as large quantities of antibiotics and / or heavy metals (zinc and copper) and high bacterial densities has been shown to promote the spread of AMR. For precautionary reasons, a broad package of measures is needed to reduce the potential risks of the spread of ARB and antibiotic resistance genes in the environment. We proposing seven options for action – including the technical upgrade of wastewater treatment plants, linking the quantities of antibiotics used in the animal house with the documentation of manure parameters, the transfer of this information from manure production via fermentation to application on soils, as well as systematic monitoring of antibiotics and AMR in the environment [3]. Thus, the occurrence and dissemination of AMR in the environment, in terms of the "One-Health" approach, can be limited. BVL and PEG (2016) GERMAP 2015 – Bericht über den Antibiotikaverbrauch und die Verbreitung von Antibiotikaresistenzen in der Human-und Veterinärmedizin in Deutschland. BVL (2017). Erneut weniger Antibiotika an Tierärzte abgegeben, Erscheinungsdatum 13.09.2017. Pressemitteilung BVL. Umweltbundesamt (2018) Antibiotics and Antibiotic Resistances in the Environment - Background, Challenges and Options for Action. https://www.umweltbundesamt.de/publikationen/antibiotics-antibiotic-resistances-in-the

MO237
The accessibility of data on environmental risk assessment of pharmaceuticals - the marketing authorization procedure in conflict with the international right of access to environmental information
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The study examines the availability of environmental information on pharmaceuticals from the point of view of the active and passive information obligation established in the Aarhus Convention. In the EU, since 1998 (veterinary) and 2006 (human) pharmaceuticals have to be assessed for their environmental risks in the context of the marketing authorization. Nevertheless, most of the active pharmaceutical ingredients (API) on the market are authorized before this time ("old" APIs), thus there is a data gap on environmental risk assessments. For some years now, parts of the environmental information generated during the procedure of marketing authorization have also been published as part of the Public Assessment Report (PAR). This publicly accessible information does not sufficiently reflect the information from environmental risk assessments. The environmental risk assessments including their effect data are not publicly available. They are treated as confidential commercial or industrial information. Therefore, even environmental authorities, like the German Environment Agency, can't use the environmental information generated by the pharmaceutical companies to compile environmental quality standards. This is incompatible with Article 4 of the Aarhus Convention and the European and national implementing provisions for this article, which require access to such environmental information for everyone. The study concludes that this contradiction can only be resolved through a publicly accessible database of active pharmaceutical ingredients and their environmental risk assessment (including effect data). This would be the basis for a monograph system, as under the EU REACH Regulation and the Biocidal Products Regulation. The database should also be extended to "old" APIs for which environmental risk assessments have not yet been carried out.

The Overlooked Hazard of Small Creeks - Elucidating the Input and Fate of Organic (Micro-)pollutants in Streams (P)

MO238
How to design sampling approaches for deriving valid degradation rates of organic micropollutants in rivers
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Anthropogenic organic micropollutants enter river systems mainly via wastewater treatment plants (WWTP). The characterization of turnover processes and fate of these compounds including also hydrological processes remains challenging in the field. This study will show the significant influence of the chosen sampling concept on derived degradation rates of organic micropollutants in river systems. Field investigations took place at two sequential reaches of the Ammer River close to Tübingen (Germany) in the southwest of Germany. Time-series measurements of the electrical conductivity (EC), conservative ions and organic chemical compounds (target screening) were conducted over a period of 24 hours, capturing the diurnal cycle of an upstream WWTP. A lumped parameter model approach including hydrological transport processes (advection and dispersion) was applied to derive degradation rate constants from comparing the same water parcel upstream and downstream of one reach. Calculating degradation rates from arbitrary grab samples entering and leaving the same reach led to different results. Dilution caused by tributaries and groundwater inflow was considered in both approaches. The difference in estimated degradation rates underlines their dependence on identifying and including hydrological boundary conditions of river systems and input dynamics of WWTPs.

MO239
Impacts of hydrological dynamics on the fate of pharmaceuticals in a small river - a model-based analysis
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In recent years, emerging micropollutants, e.g. pharmaceuticals, at concentrations in the range of nano- to micrograms per liter have become a major concern for river water quality in industrialized countries. Since the fate and effects of these compounds in the environment are not completely understood model-based analysis of field experiments can help to elucidate their accumulation and attenuation processes in rivers. We thus developed a one-dimensional reactive solute transport model coupled with transient storage and applied it to the Steinlach River, a small river impacted by a WWTP in southwest Germany, to investigate the fate of representative pharmaceuticals and the effects of transient storage on pollutant removal. Taking advantage of tracer experiments and a Lagrangian sampling campaign performed both at day and night, the change of transient storage with changing flow rates was quantified and attenuation mechanisms for different pharmaceuticals were differentiated. Results show that transient storage plays a significant role for flow and solute transport in rivers, especially under low-flow conditions. The increase in transient storage ratio increases the travel time and thus affects solute transport. However, when river discharge is high, transient storage can be negligible. The studied river segment of the Steinlach River is 1310 meters long. While carbamazepine is relatively conservative along this section, sulfamethoxazole which is biodegradable shows a daily mean removal of around 13%. Metoprolol and venlafaxine undergo both photo- and bio-degradation leading to a daily mean removal of 23-26%. Degradation processes are substantially affected by local conditions, e.g. pollutant removal decreases with increasing flow rates. This influence is especially significant for pollutants that undergo biodegradation only. The model presented in this study provides a good tool to understand the fate of emerging contaminants and identify impacts of hydrological conditions on pollutant attenuation along rivers.

MO240
NAWA SPEZ 2017: Chemical and bioassay based risk assessment of pesticide mixture in Swiss streams for aquatic plants
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Small streams represent 75% of the Swiss river network, but information about pesticide contamination as well as possible ecotoxicological effects in agricultural areas is still limited. To investigate the situation in Swiss streams, a special monitoring program (SPEZ) was conducted on five streams as part of the National Surface Water Quality Monitoring Network (NAWA) from March to October 2017. Composite 3.5-day water samples were taken continuously from March to October 2017 using automated sampling devices. Samples were extracted using solid phase extraction (SPE) and were subsequently analysed for over 200 organic pesticides using HPLC-HRMS. Additionally water samples or SPE extracts were analysed with bioassays to complement the chemical risks assessment. In a first step, we calculated acute mixture risks for aquatic plants based on the measured concentration (MEC) and the corresponding environmental quality standard (EQS). To calculate mixture risks we added the risk quotients (RQ= MEC/EQS) of all substances for which effect concentrations of aquatic plants were within a factor of 10 of the most sensitive species. At four out of five sampling sites at least one sample had a mixture RQ > 1, with a maximum RQ of 11. In a second step, we tested the ecotoxicological effects of the water samples using the combined algae assay using a green algal species *Raphidocelis subcapitata*. The Test endpoints were PS II inhibition (after 2h) and algal growth inhibition (after 24 h). Results were calculated as diuron equivalent concentrations (DEQ). Using the EQS for diuron, DEQs can thus be used to calculate bioassay based RQ. The combined algae assay showed elevated mixture RQ at the same sites where RQ >1 based on analytical data. Some of the tested water samples induced very high effects in the combined algae assay, resulting in an RQ of up to 17. In most cases, both methods correlated well, with the combined algae assay indicating slightly

higher risks. At one sampling site, the bioassay based RQ was up to 10 times higher than the chemical based RQ, which suggests the presence of undetected substances. Only when mixture risk was dominated by substances for which aquatic plants other than green algae were most sensitive, the chemical based mixture RQ were higher. Due to the high accuracy and the ease of handling the combined algae test it has proved to be a valuable tool to experimentally assess the mixture risk of pesticides for aquatic plants in environmental monitoring campaigns.

MO241

Spatial and temporal variability of pesticides in an agricultural catchment

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Small streams are highly connected to their surrounding landscape and catchment alterations can immediately propagate to stream environments due to this close connection. As a consequence, pesticide inputs following applications of plant protection products at adjacent fields can pose a serious risk to aquatic organisms in streams that drain agricultural catchments. Inputs of pesticides can vary temporally and spatially within the catchment due to the heterogeneity of crops being cultivated in the catchment and the heterogeneity of bank structure along the stream course. Additionally, depending on the substance properties, in-stream processing of active substances affect their individual fate and transport along the stream course. We performed a two month sampling campaign monitoring 68 active substances of plant protection products to assess how the number and concentration of active substances vary temporally and along the stream in an agricultural catchment (~59 km²). The catchment investigated is characterized by a gradient of increasing agricultural land use intensity and lower contribution of near natural stream reaches from upper to the lower catchment areas. We applied a combined approach including time integrated sampling and high resolution active sampling. At six stations along the stream course passive sampler devices (Chemcatcher housing, SDB-RPS disk, PES membrane) were installed for three sampling intervals (three weeks each) to assess time integrated substance concentrations. Additionally, at two stations along the stream reach daily mixed samples (1 hour sampling interval) were collected using automatic water samplers. Active substances were analyzed with LC-MS/MS in extracts of passive sampler devices and in the water samples. Active substances with different functions (fungicides, herbicides and insecticides) were detected along the stream reach. In extracts of passive sampler devices 34 of 68 substances analyzed were detected. Moreover, the results indicated a tendency of increasing number and an overall increase of sum concentration of active substances, as well as increasing concentration of some individual substances along the stream course.

MO242

Targeted and non-targeted LC-MS workflows for the identification of dominant input sources and transformation processes of emerging pollutants in surface waters of a small but complex river system

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Emerging (micro)pollutants and their metabolites are easily overlooked during routine environmental monitoring programs if they are not covered by existing water-quality regulations. The potentially high pollutant concentrations in small river systems due to a limited dilution power in relation to emissions from wastewater treatment plants and diffuse sources may pose a higher risk for living organisms there. Highly sensitive and selective analytical techniques are required for the challenging identification of the large number of mostly unknown compounds, complex matrices and low concentrations in the aquatic environment. For this reason, mass spectrometric (MS) techniques including low and particularly high resolution (HR) mass analyzers coupled to liquid chromatography (LC) are the method of choice to investigate river waters. The study aims to identify dominant input sources of micropollutants and to understand *in-stream* attenuation of micropollutants in a small but complex river system over distance and time. Therefore, surface water of the Ammer River, a tributary of the Neckar River close to Tübingen (South-west Germany), was investigated by Lagrangian sampling. A representative river segment was chosen that combines two reaches with different river morphologies and all existing/identifiable tributaries and ground water inflows. Samples were taken as grab samples or over 24 h at a temporal resolution of 1 h/6 h. A LC-QqQ-MS/MS target method was established and validated for the quantification of about 88 relevant organic pollutants including mainly pesticides and pharmaceuticals as well as their metabolites. Non-target screening data sets were generated by LC-QTOF-HRMS measurements. Generic data evaluation workflows were developed that allow the identification of unique input sources by applying logical correlations. Focusing on persistent pollutant metabolites, a comprehensive screening approach for about 60 abiotic and biotic transformation processes by an HR-mass shift driven procedure and Kendrick mass analysis was designed. In addition, a suspect screening for database-predicted transformation products of possible target analytes was included. In conclusion, an integrated approach of target, suspect and non-target techniques with emphasis on LC coupled to MS and unique workflow strategies revealed dominant input sources and relevant attenuation processes of emerging pollutants in the Ammer River.

MO243

Uptake calibration and modelling of Chemcatcher passive samplers for polar organic compounds

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Passive samplers are easy-to-use and cheap alternatives to grab sampling for monitoring of waterborne micropollutants. The compounds are preconcentrated on a sorbent material and eluted in the laboratory after retrieval from the field. The range of target compounds mainly depends on the sampling phase used. A relatively new sorbent disk, the Atlantic HLB Disk, contains HLB (hydrophilic lipophilic balance) material which is fixed in a glassfibre body. It can be used in the modular designed Chemcatcher. For the calculation of time-weighted average water concentrations from the accumulated amounts of chemicals in the samplers, passive samplers need to be calibrated in the laboratory. Uptake kinetic was measured in a three week calibration experiment in a flow-through tank at 15 °C with polar pesticides and pharmaceuticals. The uptake results were fitted using different models (linear and nonlinear) and the influence of a diffusion limiting membrane was considered. Partitioning coefficients between water and sampling phase could also be derived from the models and were compared with results from batch equilibration experiments.

Micro(Nano)plastic Pollution: Tackling the Plastic Problem by Identifying Sources, Investigating Fate and Novel Approaches (P)

MO244

Emissions and mitigation of synthetic microfibres from machine washings and tumble dryings

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Synthetic microfibres released in cloth washing are assessed to be one of the major sources of primary microplastics into the environment. The annual emission of polyester microfibres from household washing machines has been estimated to be 150,000 kg in a country with population of 5.5×10^6 (Finland). The objectives of this study were 1) to quantify the emissions of synthetic textile fibres discharged from sequential machine washings and tumble dryings and 2) to determine the collection efficiency of two commercially available microplastic traps: washing bag (GuppyFriend) and washing ball (Coraball). The synthetic fabrics were four types of polyester textiles (two fleece fabrics, one softshell fabric and technical T-shirt), one technical T-shirt of polyamide and one jersey of polyacryl. All the fabrics were new/unused and they were separately treated in five sequential washing-drying cycles. For the assessment of microplastic traps, the new/unused and identical polyester technical T-shirts were washed as three replicates with and without the trap under the investigation. The replicate samples from total washing waters were filtrated and subsequently analysed gravimetrically and under an optical microscopy. The fibres released in the tumble drier were collected after each drying and their masses were determined. The number and mass of microfibers released from test fabrics in the first wash varied in the range 1.0×10^5 to 6.3×10^6 kg^{-1}, and 0.038 to 0.22 % w/w, respectively. The corresponding mass range in the first drying was 0.001 to 0.17% w/w. Fibre emissions showed a decreasing trend both in sequential washes and dryings. The ratio of machine washing to tumble drying varied between the fabrics: the ratio was nearly one or lower to polyester fleece fabrics whereas it was much larger to other tested textiles. GuppyFriend washing bag trapped approximately 30 % of the polyester microfibres discharged in washings whereas Cora Ball did not mitigate the emissions of microplastics on a mass basis. The sampling, sample pretreatment and identification/quantification methods have to be developed in order to get reliable information on the occurrence, abundance and environmental fate of these synthetic microplastics.

MO245

Synthetic microfibres and small microplastics in wastewater effluent

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The domestic washing of textiles is a major source of synthetic microfibres entering a wastewater treatment plant. It has been estimated that the annual emission of the most common synthetic fibre, polyester, from households is approximately 154,000 kg in Finland (population $5.5x10^6$). In earlier studies, wastewater treatment processes have been shown to remove effectively microplastics from wastewater influent to sewage sludge, but the synthetic microfibres have not been in the main focus of the studies due to the large volume samplings and high contamination risk. Consequently, the efficiency of wastewater treatment processes for synthetic microfibres and small microplastics is still poorly known. The objectives of this study are 1) to develop and optimise the sampling and pretreatment methods for low volume wastewater effluent samples and 2) to investigate the temporal variation of small microplastics in the effluent. Particular effort is put to mitigate and assess the sample contamination. The samplings are carried out as three replicates of 2 litre grab samples. The laboratory and field blank samples are handled identically with the effluent samples. The chemical and possible enzymatic sample pretreatment steps are conducted in a laminar flow hood. The sample preparation steps are optimised for an efficient removal of organic matter. Microplastics with the smallest dimension of ≥10 µm are identified by using a Fourier Transformation Infrared microscope (µFTIR; Spotlight 200i, Perkin Elmer). The number concentration of synthetic microfibres and microplastics are shown and the diurnal variation in number concentrations is discussed in the presentation.

MO246

Microplastic fluxes from a Swedish wastewater treatment plant

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Waste from households, industries and urban run-off is usually collected in sewer systems. Large quantities of microplastics (MP) can be found in wastewater e.g. from MP in cosmetics or personal care products, release of synthetic fibers from washing of clothes, MP in urban runoff from air deposition or weathering of plastic materials. This creates a concentrated stream that needs to be treated before it can be discharged to a recipient. Recent publications show that wastewater treatment plants are effective in removing microplastics. It is suggested that the treatment processes lead to a concentration of the MPs in the sludge. Due to its high content of organic material, sludge is often used as a fertilizer on agricultural fields leading to a potential spreading of MP to the terrestrial environment. In this study, inlet samples as well as samples from coarse and fine screens (20 mm and 2 mm), digested sludge and effluent water was analyzed using µFTIR-imaging and ATR-FTIR. The grit samples were first sterilized using 10 % H_2O_2. Afterwards plastic-like particles were visually identified and sorted out. Before analysis, all other samples underwent a multistep sample preparation consisting of pre-treatment with SDS and H_2O_2, followed by enzymatic digestion, iron catalyzed oxidation with H_2O_2 (Fentons reaction) and, at last, density separation with $ZnCl_2$ at >1.7 g/cm^3. After sample preparation the samples were split in two fractions according to size: above and below 500 µm. MP particles above 500 µm, as well as those from the grit samples, were analyzed with ATR-FTIR. The smaller size fraction was concentrated in 5 ml of 50% ethanol from which a subsample was deposited on a ZnSe window and analyzed using FPA-µFTIR-imaging. MP was automatically identified from the acquired IR-map using our in-house software, MPhunter.

MO247

Occurrence of microplastics in municipal sewage treatment plant(STPs) in Korea

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Municipal sewage treatment plants (STPs) are suspected to be major point sources of microplastics in freshwater systems. In order to know the occurrence of microplastics in Korean STPs, a preliminary nationwide monitoring of microplastics in STP influents and effluents was conducted in 50 STPs covering wide range of treatment capacity over the country. Triplicate samples of 20 mL influents were filtered through 100 µm filter and 1000 L effluents were passed through a custom-made sampling sieve (pore size of 100 µm). Filtrates were further treated using wet peroxidation using hydrogen peroxide and density separation using zinc chloride solution. Isolated particles were identified under a Fourier-transform infrared spectroscopy (FT-IR) and a dissection microscope. Recovery of polyethylene particles added to influent samples was over 85%, supporting that the method is acceptable for monitoring microplastics. Thermoplastics and synthetic fiber were main materials of microplastics in STPs. Typical number concentration ranged 200-1,000 particles per liter in influents and 0.01-0.3 particles per liter in effluents. Daily microplastic flow to STPs was estimated approximately 200,000 particles per capita-day, close to recent assessment in other countries. Removal efficiency of microplastics in STPs were mostly greater than 99.9%. In addition, it was found that typical anaerobic/anoxic/oxic (A2O) processes remove incoming microplastics significantly better than biocube sponge processes. Particle size distribution of isolated microplastics followed a power law relationship down to the cutoff size (100 mm), implying that majority of microplastics are secondary microplastics.

MO248

Microplastics loading estimation from urban areas to sewage treatment plants based from a monitoring campaign in Japan

Y. Kameda, Chiba Institute of Technology / Creative Engineering

One of a main land-based sources of micropalstics (MPs) released to aquatic environment is considered to be human activities such as our daily life, industrial activities, agricultural activities and so on. These MPs are released to wastewater and runoff water from sources and finally flow into sewage treatment plants (STWs). To control the concentration in surface water, it is important to estimate the load from urban areas to STWs and reveal the removal rates at STWs. In this study, a MPs monitoring campaign was performed in order to measure the concentration in raw wastewater samples and effluent water samples at approximately 20 STPs in Japan. The concentration variations in raw wastewater samples during a day were also investigated. MPs in raw wastewater and effluent water samples were analyzed by our novel analytical method “automatic microFTIR with Multivariate Curve Resolution analysis and Correlation analysis”. Each polymer type microplastics less than 100µm are qualified and quantified into each shape types such as fragments, spheres, fibers and so on. Based on these data, microplastics loading per capita to STWs can be estimated statistically. Removal rates are also estimated at Japanese STPs. These data will be used to simulate their concentrations in rivers and bays near large cities by simulation models. This presentation will show some results in this monitoring campaign.

MO249

Microplastic in Biosolids and Agriculture Soils Receiving it as Fertilizer

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Microplastics (MP) have been measured in large abundance in wastewater. Recent studies showed that wastewater treatment plants effectively retain MP, since low MP concentrations are measured in the effluent compared to the influent. However, this indicates that MP likely accumulates in the wastewater sludge. This sludge has a high content of organic material and phosphorous and is hence commonly used as agricultural fertilizer. This use of sludge could potential cause spreading of MP to the terrestrial environment, a process that hitherto has received little attention. This study focuses on the abundance of MP in the size range 5000-10 µm in agriculture soils that have received wastewater sludge as fertilizer (biosolids) for the last 35 years. It presents a protocol of sample preparation as well as field data. In Malmö, Sweden, three fields were sampled. One field had received 3 tons/ha of biosolids, another 1 ton/ha and one had received mineral fertilizer only. From each field, 40 kg of soil were sampled from the top soil layer and brought back to the lab for analysis. The microplastic concentration was in general low. Hence the soil had to undergo extensive treatment to extract the plastic particles, and protocols were developed for this purpose. Upon extraction the MP were analysed on a state-of-the-art FPA-µFTIR-Imaging system (10-500 µm MP) and ATR-FTIR (500-5000 µm MP). The FTIR-analysis showed the following MP concentration-tendency; Soil with 3 tons/ha > soil with 1 ton/ha > soil receiving mineral fertilizer only. However, the detected MP concentration in the soil fertilized with a biosolids load of 1 ton/ha and the soil receiving no biosolids were rather close. Overall, the results showed that more plastic types were found in the soil fertilized with biosolids compared to the soil that received mineral fertilizer only. This indicates that the composition of MP in soil may have been affected by the biosolids load.

MO250

Occurrence of fine microplastics and other emerging debris in Lake Bikal

Y. Kameda, Chiba Institute of Technology / Creative Engineering; M. Yamamuro, Tokyo Unversity; M.V. Moore, Wellesley College; O.A. Timoshkin, Limnological Institute SD RAS

Lake Baikal is the largest freshwater lake by volume in the world. There are many researches about chemical contaminants in the lake such as PAHs, PCBs, Dioxins and so on. However there are few researches about microplastics (MPs). This psesentation will reveal occurrence of MPs in Lake Bikal. The surface water samples were collected and were passed through 10 µm nets. MPs in the collected particles were extracted by H_2O_2 digestion and NaI density separation. The MPs on a membrane were identified and quantified by our novel analytical method “automatic microFTIR with Multivariate Curve Resolution analysis and Correlation analysis”. This presentation will show tha characteristics of MPs in Lake Bikal.

MO251

Occurrence of fine microplastics and other emerging debris from a river in Japanese urban area

Y. Kameda, Chiba Institute of Technology / Creative Engineering

An occurrence of microplastics (MPs) was investigated in contaminated river water located in a Japanese metropolitan area. The surface water samples were collected at 5 sites from the upstream site to the downstream area. The collected samples were passed through 10 µm nets. MPs in the collected particles were extracted by H_2O_2 digestion and NaI density separation. The MPs on a membrane were identified and quantified by our novel analytical method "automatic microFTIR with Multivariate Curve Resolution analysis and Correlation analysis". By our methods, many fine MPs whose sizes ranged from 10 µm to 100 µm were observed. The concentration was over one thousand particles in 1 m^3 which was much higher than those in previous studies. The changes of the each polymer concentration from upstream to downstream were also evaluated as well as the impact of release from sewage treatment plants and runoff water from a city area. In the middle reach site, a large amount of white fibers were found with MPs. These fibers were identified as asbestos by the analysis method. The origin of them was estimated to be building construction site near the river. This presentation reveals environmental behaviors of ordinary large MPs as well as fine MPs and new emerging debris in the polluted river.

MO252
First evidence of microplastics in the sediments of the Limfjord: hydrodynamic modelling-based sampling combined with FPA-µFTIR-Imaging automated analysis

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Microplastic (MP) pollution is a global concern affecting almost the totality of the water bodies, including transitional environments such as lagoons, salt marshes, and estuarine areas. Monitoring activities are hence paramount to fill knowledge gaps on MP occurrence and spatial distribution. Therefore, we conducted the first MP sediment monitoring survey of the Limfjord, the largest Danish fjord (1500 km^2). Its average depth is 4.9 m, with a freshwater average inflow of 2.7 km^3 y^{-1} and a net flow of 6.8 km^3 y^{-1} passing from west to east due to the wind and tidal impacts. The total catchment area (7600 km^2) includes Aalborg (~130000 inhabitants) and other smaller cities, harbours and shellfish farms along the shores. The sampling locations were selected taking into account the hydrodynamical conditions and considering the bed shear stress induced by the currents as the main parameter for sedimentation, erosion, and resuspension of materials. The shear stress was modelled with a calibrated non-steady hydrodynamic model (MIKE3FM), including both effects of time-varying boundary conditions, stratified conditions due to salinity gradients and wave-induced currents based on a spectral wave calculation. Sampling was conducted in areas with low bed shear stresses, where the light material is expected to settle, also considering potential direct MP sources and sampling both up and downstream of the major city (Aalborg), WWTPs and riverine inputs. Samples were collected with a Van Veen grab, processed by flotation ($ZnCl_2$) and multi-step sample clean-up (enzymatic treatment, Fenton reaction), then analyzed by FPA-μFTIR-Imaging spectroscopy/MP auto-analysis (MPhunter). Preliminary results showed a diffused MP contamination (600-4000 part. kg^{-1} and 50-900 μg kg^{-1}), highlighting higher concentration at the urban stations and downstream the city, but highly depending on the hydrodynamic conditions (location with higher bed shear stress showed a lower MP abundance). The polymer composition showed mainly polyethylene (25%), polypropylene (26%), polyester (17%), and polystyrene (17%). Although the MP concentration cannot be directly correlated to the mud % and to the shear bottom stress in a dynamic environment like the Limfjord, the combination physical systems' understanding (e.g. throughout modelling) and unbiased analytical measurements provides powerful tools to improve data quality and, therefore, a better understanding of MP spatial distribution.

MO253
Occurrence of fine microplastics and other emerging debris in aquatic biota at a contaminated bay compared with those in Japanese tropical area

Y. Kameda, Chiba Institute of Technology / Creative Engineering

An occurrence of microplastics (MPs) was investigated in oysters in a polluted large bay, Tokyo bay compared to beaches in Okinawa, tropical island in Japan. The oyster samples were collected at several points along the Tokyo bay and several areas at Okinawa. The collected samples were digested by KOH and extracted by NaI density separation. After the separation, H_2O_2 digestion was performed. Finally the particles were collected on a membrane. The MPs on a membrane were identified and quantified by our novel analytical method "automatic microFTIR with Multivariate Curve Resolution analysis and Correlation analysis". By our methods, many fine MPs whose sizes ranged from 10 µm to 100 µm were observed. The MPs profiles were different among sites even in Tokyo bay. These results indicated that some of MPs in oysters might be originated from land-based MPs or those from sewage treatment plants. A large amount of alumina particles and other particles were also identified in oysters by our method. These new particles are not measured in recent researches. Their toxicity and ecological risk assessment will be necessary and this method is a powerful tool for non-target analysis of particles in aquatic organisms.

MO254
Understanding sources and transport of microplastic pollution to the Canadian Arctic

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Evidence continues to emerge showing the ubiquitous distributions of microplastics in the environment, and there is growing evidence showing their presence in remote Arctic environments. The presence and impact of microplastics in the Arctic may be exacerbated by climate change, due to increased ship transportation through the north-west passage, changing air and ocean circulation, and release of microplastics from melting of multi-year sea ice. Our research aims to determine the extent of microplastic contamination across the Canadian Arctic archipelago and Hudson Bay marine systems, and to evaluate sources and transport pathways of the microplastics. Fourteen surface sediment (0-5 cm) samples were collected between 2014 and 2017 across approximately 3,300 km of the Canadian Arctic archipelago (CAA) marine system. An additional seven surface sediment samples were collected from Hudson Bay in 2018. Sediments were collected from water column depths ranging from 40 to ~1500 m using a box corer, as part of the Northern Contaminants Program during yearly ArcticNet expeditions. Sediments were examined for microplastic particles > 20 μm, specifically particle abundance, distribution and characteristics. Field and laboratory blanks were collected and processed concurrently. Our data indicate the presence of microplastic particles in all 14 CAA samples. However, there was great spatial heterogeneity of abundances; blank-corrected counts varied by an order of magnitude across sites, from 700 to 6,700 particles/kg dried sediment. Blanks contained 0-5 particles/sample, all of which were fibers. The dominant particle type in samples was fibers, ranging from 60 to 100% of particles per sample. The greatest abundances of microparticles were found in the western Arctic offshore of Alaska in the Beaufort Sea, and in the deposition zone of the Mackenzie River, Northwest Territories, as well as in the eastern Arctic surrounding Baffin Island and in the Davis Strait. Our results indicate the ubiquity of microplastics across the Canadian Arctic marine systems, and that microplastics likely undergo long-range transport via oceanic and air currents, and riverine systems to reach the Arctic. In addition, local sources may contribute to microplastic abundance, even in remote environments.

MO255
Occurrence and distribution of metals in plastic litter from Spanish Mediterranean coasts

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Marine litter is a global problem, and is known to negatively affect the environment in several ways. Less understood, however, is the role of plastics in providing a vector for exposure to and accumulation of chemicals that are adsorbed to or incorporated within the polymeric matrix. Moreover, the majority of studies have targeted persistent organic pollutants, but very little attention has been paid to the occurrence and impacts of metals in marine litter [1]. Once released into the marine environment, plastic litter inmediately begins to undergo a variety of weathering processes. The process is influenced by polymer type and the presence of additive chemicals such as metals and metalloids, currently used as catalysts, biocides, pigments for colour and UV and heat stabilisers [2]. In this study, plastic litter was sampled at three Iberian Peninsula Southeastern beaches (Calblanque, Cape Cope and La Llana), each affected by different predominant anthropogenic activities (tourism, agriculture, urban, etc). Plastic litter was characterized by ATR-FTIR, and RX fluorescence was used as a semiquantitative technique that indicates the main metals contained in the samples. PE and PP were the most abundant polymers present at the plastic litter in two coastal areas (Cape Cope and Calblanque) whereas PA was the predominant one in La Llana. The presence of Ca, Cl, Al, Fe and Ti has been found in most of the analyzed samples. From the results here shown it is clear that plastics present at marine litter constitute a pollution vector, but our findings highlight the need for further studies and because of that we are studing the desorption of these pollutants for the first 24 hours to seawater to estimate their potential transfer to the seawater as it has been previously determined for organic contaminants [3]. REFERENCES [1] Turner A. 2016. Heavy metals, metalloids and other hazardous elements in marine plastic litter. Mar. Pollut. Bull. 111:136-142. [2] Kühn S *et al.* 2018. Marine microplastic: Preparation of relevant test materials for laboratory assessment of ecosystem impacts. Chemosphere. 213:103-113. [3] León V.M. *et al.* 2018. Potential transfer of organic pollutants from littoral plastics debris to the marine environment. Environmental Pollution 236, 442-453. *Acknowledgement* - Xunta de Galicia, partially financed by ERDF (ref ED431C 2017/28). Ministry of Economy and Competitiveness: PCIN-2015-170-C02-01 - BASEMAN (JPI Oceans), CTM2016-77945-C3-3-R (ARPA-ACUA)

MO256
Microplastics in Santa Catarina Island, Southern Brazil: occurrence, distribution and characterization

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Once in the marine environment, plastics represent a global concern with ecological, health and economic consequences. Plastics causes entanglements, act as a transport vector for contaminants, and cause large financial costs through lost tourism. In Brazil, several studies reported different-size plastics on sandy beaches, but there is no data regarding microplastics in Santa Catarina Island. Therefore, in the present study microplastics (0.5 - 5 mm) were quantified and characterized by FTIR analyis in ten beaches from Santa Catarina Island, an important area for tourism and mariculture. A total of 572 particles classified as fragments (47%), pellets (37%), fibers (2%) or styrofoam (14)%. Concentrations ranged from no items to 36 itens m-2 and from 1.32 to 126 itens m-2 in the backshore and strandline, respectively. Northern Moçambique was the place with the greater concentrations (126 and 64 itens m-2, respectively). Since Northern Moçambique, located inside a State Park, is neither a popular place for tourism nor for the practice of aquatic sports the highest values of debris could only be explained by physical features and/or beach dynamics. Northern Moçambique was the beach with the largest concentration of mesoplastics, follow by Brava > Pântano do Sul > Lagoinha do Norte > Ribeirão da Ilha > Barra da Lagoa > Joquina> Sanbaqui ~ Solidão. Lagoinha do Norte > Brava > Ribeirão da Ilha > Sambaqui ~ Southern Moçambique > Pântano > Solidão ~Sambaqui. Accumulation of mesoplastic can be affected by physical features, beach dynamics, proximity to urban centers and presence of beach goers. Since Northern Moçambique, located in the northern portion of Rio Vermelho State Park, is neither a popular place for tourism nor for the practice of aquatic sports the highest values of debris could only be explained by physical features and/or beach dynamics. On the other hand, both Solidão and Sambaqui are well protected beaches with low urbanization.

MO257
Characterization of microplastics in sand of the Concepción bay, central Chile
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In recent years, there has been increasing concern about "microplastic particles" (MP) (plastic fragments smaller than 5 mm) in the environment. Due to their small size, MP are considered bioavailable for organisms throughout the food chain and for humans. In Chile, recent studies have detected microplastic in abiotic matrices along the Chilean coast. In this study we assess the occurrence of MP in sand samples from Concepcion Bay which is a coastal ecosystem with a vital role in the economic sustainability of the Biobío region of central Chile. The bay holds several beaches, along the coast, used for recreational activities by the population during the summer period. In this study we have characterized MP, in sand, of the beaches of Concepción bay, in central Chile, during two years in different seasons in order to preliminary assess population exposure to MP. Samples of MP were collected and analyzed physically and chemically. Samples (250 g) were collected in four location of the bay, six beaches: i) Dichato, ii) Coliumo, iii) Tome (Bellavista, Morro, Estación) and iv) Penco, during four seasons (September 2015 (winter) - March 2018 (Summer)). Samples were taken in glass bottles and analyzed using a saline solution (NaCl 1.2 kg/L) and filtered using a filtration system under vacuum, with glassfiber filters (Whatman GFF). Then, the samples were analyzed physically (size, colour and shape) using optical microscope with an integrated camera, Leica DM 750 and chemically screened under a FT-IR spectrometer Agilent Cary 630. The preliminary results of MP in sand samples showed, in general, an average abundance of 0.07 ± 0.04 MP items/g, showing greater abundance in summer season. The physical characterization showed the predominance of MP < 0.5 mm in the different sampling sites as follow: Coliumo beach (67%), Tome - Estación (50%) and Dichato (67%)); the most abundante MP shape was fiber (Bellavista (72%), Estación (50%) and in the Morro beach (54%)); solid MP pieces accounted for 34% for Coliumo and 67% for Dichato. The colour with the highest frequency of detection was red colour (detected at Coliumo (50%), Bellavista (43%), Estación (50%) and Dichato (38%)), and black colour (Penco (60%) and Morro (46%)). The polymer analysis showed that the most frequent plastics identified were polypropylene. These data are the first information of MP in Concepción Bay and are consistent with other studies in the scientific literature.

MO258
Occurrence of fine microplastics and other emerging debris in seawater from Japanese tropical area
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An occurrence of microplastics (MPs) was investigated in seawater in a contaminated bay, Tokyo bay compared to that in Okinawa, a tropical island in Japan. The seawater samples were collected and passed through 10 µm nets . MPs in the collected particles were extracted by H_2O_2 digestion and NaI density separation. The MPs on a membrane were identified and quantified by our novel analytical method "automatic microFTIR with Multivariate Curve Resolution analysis and Correlation analysis". Amount of large plastics debris comes to Okinawa coastal area from China and other Asian countries. But many MPs in seawater at beach were black fibers in a beach. These MPs were identified polyvinyl alcohol and similar to nets used by fishermen. Many particles were identified as salt of stearic acid, whose log Kow is 4.13. This method could detect many small MPs which were not identified by visual inspection.

MO259
Investigating the effect of urbanisation on microplastic pollution, water quality and pollutant sensitivity of Daphnia magna: a case study in pond surface waters of Birmingham, UK
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Freshwater habitats have been shown to accumulate concentrations of microplastics (MPs) equalling or exceeding those reported for the marine environment. Due to the variation of potential point (wastewater) and diffuse (road run-off) sources of MPs into the urban aquatic environment, the presence and sensitivity of keystone species to MPs within this ecosystem is of great interest. \n A study conducted at 15 ponds spanning an urban-rural gradient in and around Birmingham, UK aimed to address the following aspects of MP freshwater pollution: (1) the scale and characteristics of freshwater MP pollution by assessing the types and sizes of MPs present, (2) the water quality of the sites determined through parameters such as dissolved oxygen and the presence of macronutrients, and (3) the abundance of *Daphniidae* and their sensitivity to further pollutant exposure following acclimatisation in the lab. Environmental parameters such as pH, temperature, conductivity and flow rate were taken at each pond. \n MPs were classified by morphology (e.g. fragment, fibre, spherule, foam, sheet), size, colour and polymer type using microscopy techniques and Fourier-Transform Infrared Spectroscopy (FTIR). From the invertebrate samples, *Daphnia magna* were identified and counted before being cultured in borehole water for several generations prior to EC_{50} determination following exposure to sodium dodecyl sulfate and 1 μm carboxylate-modified polystyrene MPs individually and as mixtures. These were conducted to ascertain if the daphnids' sensitivity to pollutants varied depending on the level of urbanisation and pre-exposure to MPs in the locations they were collected from. Water quality was assessed and compared to current standard media (e.g. HH COMBO) for daphnids in terms of the macronutrient availability determined using ICP-MS analysis of the major ions. Field parameters combined with suspended solids and ion concentrations allowed further understanding of the variability of water quality across the sampled sites. \n Combining the various elements allows us to hypothesise the links between water quality and growth conditions of *Daphnia* in their natural habitat and how their environmental history can affect their sensitivity to subsequent pollutant exposures even after several recovery generations in clean water.

MO260
Size matters: ingestion of relatively large microplastics contaminated with environmental pollutants posed little risk for fish health and fillet quality
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In this study, we investigated biological effects associated with ingestion of polystyrene (PS) microplastic (MPs) in fish. We examined whether ingestion of contaminated PS MPs (100-400 μm) results in chemical stress in rainbow trout*(Oncorhynchus mykiss)* liver and we explored whether this exposure can affect the oxidative stability of the fillet during ice storage. Juvenile rainbow trout were fed for 4 weeks with four different experimental diets: control (1) and feeds containing virgin PS MPs (2) or PS MPs exposed to sewage (3) or harbor (4) effluent. A suite of ecotoxicological biomarkers for oxidative stress and xenobiotic-related pathways was investigated in the hepatic tissue, and included gene expression analyses and enzymatic measurements. The potential impact of MPs exposure on fillet quality was investigated in a storage trial where lipid hydroperoxides, loss of redness and development of rancid odor were assessed as indications of lipid peroxidation. Although, chemical analysis of PS MPs revealed that particles sorb environmental contaminants (e.g. PAHs, nonylphenol and alcohol ethoxylates and others), the ingestion of relatively high doses of these PS MPs did not induce adverse hepatic stress in fish liver. Apart from a small effect on redness loss in fillets, PS MPs ingestion did not affect lipid peroxidation or rancid odor development, thus did not affecting fillet's quality.

MO261
Ingestion and transfer of microplastics pre-incubated with and without crude oil in the planktonic food web
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Marine debris is a growing problem to which plastic makes a major contribution. Once released in the marine environment large plastic items can undergo several chemical and physical degradation processes. Photooxidation, weathering and mechanical fragmentation from wave action all contribute to the generation of smaller-sized particles, such as microplastics. A decrease in particle size may increase its bioavailability and its potential threat to aquatic life. Recent studies have identified several different adverse effect mechanisms. Plastic fragments can significantly alter the ecophysiology of marine organisms by clogging the gut and digestive system, inducing starvation as well as histological alteration of organs. In addition, there is growing evidence of the ability of microplastics to adsorb and act as carriers for organic contaminants, hence promoting their global distribution and accumulation in marine organisms through trophic levels. The combined effect of microplastic and absorbed contaminants ton aquatic food webs is still poorly understood. To address this, food web transfer experiments were performed by exposing brine shrimp, Artemia salina, to mixtures of artificial and natural particles. The first hypothesis tested was that brine shrimp were unable to discriminate between particle types [hypothesis 1]. The chlorophyceae algae, Dunaniella salina (3-6 μm), and Texas red stained fluorescent microbeads were used to test the hypothesis. A second line of research tested the hypothesis that microplastic particles were able to transfer adsorbed organic pollutants from microplastic to target organisms as well as promote the transfer of chemicals from one food level to another [hypothesis 2]. Northern shrimp (Pandalus borealis) larvae were fed with brine shrimps pre-incubated with microplastics spiked with oil.

MO262

Screening of HBCD Enantiomers Transported by Microplastics in Wastewater Treatment Plants

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Microplastics are commonly defined as those plastic particles whose diameters are < 5 mm. As a kind of synthetic polymers, microplastics can persist in the environment for a long time. The sorption of environmental contaminants on these microplastics may be a cause for environmental and health concern due to the possible desorption of these contaminants *in vivo*. Hexabromocyclododecane (HBCD), a commonly used flame retardant, exhibits strong hydrophobicity, and thus has a high capacity of being sorbed on hydrophobic particles such as microplastics from the aqueous phase. Despite this, the sorption and desorption behavior of HBCD enantiomers on microplastics is still largely unknown. Wastewater treatment plants (WWTPs) are considered as an important source of microplastic pollution. We have carried out an investigation on microplastics in two typical WWTPs in Hong Kong, with an aim to examine the abundance of microplastics in different treatment stages from the WWTP, and the sorption/desorption behavior of HBCD enantiomers on the microplastics present in the WWTPs. Results showed that the abundances of microplastics in the two WWTP effluents were 0.27 and 0.4 particles/L, respectively, which was in the medium level compared to other studies. The removal efficiencies of microplastics reached 86.9% and 60.4%, indicating that the treatment methods were not efficient for microplastic removal. No obvious trend or tendency was found for that the proportion of different microplastic sizes shifted in different treatment stages, indicating that the removal of microplastics did not have a preference in size. HBCD was detected in two samples from one WWTPs and the total concentrations were as high as 502.3 and 4184.4 ng/g. The concentration of HBCD carried by microplastics in the final effluent was much higher than in the settled sewage, which was opposite to HBCD distribution in the aqueous phase in the WWTP. The enantiomeric patterns of HBCD were different in the sewage and carried by microplastics, which might indicate that the HBCD stereoisomers have different behaviors in the aqueous and solid phases. For the first time, the amount of HBCD carried by microplastics was estimated in the WWTPs of Hong Kong. The study brings new insight into the fate of microplastics and the role of the vector for pollutants transportation.

MO263

EXTRACTION AND DETERMINATION OF PERSONAL CARE PRODUCTS ADSORBED ON MICROPLASTICS

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At present, images of floating garbage and beaches full of plastics have attracted media attention as a real evidence of human impacts on environment. In fact, concern about the presence of plastics in oceans and seas and the effects on aquatic biota is increasing, not only among scientists, but also on policy-makers [1]. From the huge variety of plastics which arrive to water body, microplastics (MPs) have revealed as the most concerning ones. Plastics with a size lower than 5 mm are considered as MPs and they are found, not only in waters but also in sediments or aquatic organisms of all water bodies of the globe [2]. Because of their physicochemical properties, microplastics are considered a new toxic actor for aquatic organisms. In fact, in the last years, many scientific studies have been focused in the quantification of these pollutants. Nevertheless, the possibility that other pollutants as organic molecules could be adsorbed on microplastics have been not studied enough and this phenomenon could produce that the toxicity of microplastics could be even worse. Among the pollutants that have been studied adsorbed in MPs are polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs). To our knowledge, the lack of studies about sorption of pharmaceuticals on MPs makes necessary the study of this topic. In relation to extraction techniques, the use of soxhlet, ultrasounds and solvent soaking stand out [3]. In this work, we propose an extraction method for the extraction of pharmaceutical compounds adsorbed onto MPs with the aim of studying which factor affects the most its adsorption and desorption. [1] European Commission (2018). Initial Statement by the Group of Chief Scientific Advisors. *ISBN 978-92-79-88537-2* [2] Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). *Marine pollution bulletin*, *62*, 2588. [3] Hong, S.H., Shim, W.J., Hong, L., (2017). *Analytical methods,* 9, 1361

MO264

T-shirt in a Sandwich: A novel passive sampling approach for analyzing SVOC release from clothing during laundering

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Clothing and other textiles can accumulate chemical contaminants from the environment. Chemical additives are also often added to clothing during production. Clothing has been shown to release brominated flame retardants (BFRs) and organophosphate esters (OPEs) as well as fibres into wash water during laundering. However, the proportion of chemicals bound to released fibres versus solubilized into wash water has not been investigated yet, to our knowledge. Our goals were to address the following questions: (1) What fractions of chemicals released during the washing of clothing are solubilized in wash water vs sorbed to released fibres? (2) Can silicone rubber (SR) passive samplers be used to approximate the maximum chemical fraction in textile that can be easily solubilized during washing? (3) Can the distribution of the fibre-sorbed vs easily solubilizable fraction be predicted based on physical-chemical properties of the textile material and chemicals of interest? SR has been used as an effective passive sampling device for chemical contaminants in a variety of environmental matrices. Here we show the use of a novel contact passive sampling technique to investigate chemical partitioning of BFRs and OPEs between wash water and clothing. The novel method is based on the application of a 'SR sandwich' that consists of a single layer of clothing sandwiched between two layers of SR and allowed to sample over a two-week period. We tested the sandwich using 40 cotton t-shirts worn for 24 hours by volunteers. Four strips were cut from each t-shirt and treated one of the following ways: (a) extraction for total chemical concentration prior to washing, (b) contact passive sampling using the 'SR sandwich' for the portion of the chemical contaminants that are readily releasable from the material, (c) simulated washing followed by extraction of the t-shirt strip and the water for total concentration released into water and residual concentration in the t-shirt strip, and (d) simulated washing followed by extraction of filtered wash water for solubilized chemicals that leached from the material during washing. The fraction of chemicals sorbed to released fibres was estimated by subtracting the concentration of compounds in filtered wash water from the total chemical concentration in wash water (containing fibres). These results provide evidence for the role of microfibres released during washing as conduits for chemical movement from the indoor to outdoor environment.

MO265

Adsorption behaviour of musk fragrances on LDPE microplastics

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Microplastics (MPs) and synthetic musks are organic pollutants of great concern in the marine environment.There are many sources of microplastics, including cosmetic additives, laundry wastewater, and the photolysis of large plastics, and are potential carriers of organic pollutants due to their recalcitrance and high specific surface area. On the other hand, synthetic musks have been widely manufactured and used as fragrance additives in chemical commodities for daily use, such as perfumes, cosmetics or shampoo. [1] Synthetic musks have been detected in several environmental samples and due to they are only partially biodegradable, they are not completely eliminated by wastewater treatment plants (WWTPs). To date, environmental regulatory limits have not been set for these compounds, and it is extremely important to study their occurrence in the environment, in order to determine whether they can be considered hazardous for human health and environment. [2] In this stydy, the adsorption of six synthetic musks (Celestolide, Galaxolide, Tonalide, Musk xylene, Musk moskene and Musk ketone) by low density polyethylene (LDPE) was investigated. The assessment of the changes and processes that MPs suffer during weathering is essential to understand their effects in the environment, and therefore the tests were made with pristine and weathered LDPE. The adsorption experiments were performed at room temperature (20ºC) and at 4ºC using simulated seawater prepared with sea salt. Samples were collected at 24, 48, 96 and 168 hours. The adsorption to the plastic is observed already at 24 hours. Moreover, adsorption capacity was affected by temperature which is explained because the compounds have lower solubility in water at low temperatures, and few differences are observed between the pristine plastic and the weathered one. REFERENCES [1] Zhang X *et al.* 2018. Sorption of three synthetic musks by microplastics. Mar. Pollut. Bull 126:606-609. [2] Homem V, *et al*. 2016. Ultrasound-assisted dispersive liquid-liquid microextraction for the determination of synthetic musk fragrances in aqueous matrices by gas chromatography-mass spectrometry. Talanta 148: 84-93. *Acknowledgement* - This work has been supported by Xunta de Galicia, partially financed by ERDF (reference: ED431C 2017/28). The Ministry of Economy and Competitiveness is also thanked: subproject PCIN-2015-170-C02-01 of the EU-Funded BASEMAN (JPI Oceans) and, project CTM2016-77945-C3-3-R (ARPA-ACUA)

MO266

Soil physicochemical property dependent toxicity of microplastics on nematode species

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Problems associated with plastic particles in soil ecosystems have been highlighted, and some studies have started to investigate these problems. Notwithstanding many reliable results in previous studies supported that adverse effects could be induced by nano- and micro-plastic exposure, they may not represent real soil environmental conditions because some of these studies were conducted in non-soil media such as liquid and mixtures of plaster and activated charcoal. In the present study, we conducted nematode bioassay in liquid and soil media to evaluate the media-dependent effects of nano- and micro- plastic particles. Field soil samples were used to assess whether the effects of plastic particles are dependent on particle size and soil physicochemical properties. Polystyrene beads of two different sizes (42 and 530 nm) were chosen as target materials, and the target organism, *Caenorhabditis elegan*s, was exposed to liquid and soil media containing each PS beads. We performed same bioassays using five different field soil samples with different physicochemical properties. The soil media including plastic particles was more toxic system than liquid media, and plastic size-dependent toxicity were only observed in soil media. As result on field soil samples, we also found that soil texture composition can be highly related with plastic toxicity. This is first study reporting reliable quantification of plastic toxicity and its interaction with soil properties, and will provide the important knowledge on plastic toxicity in soil system *This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2016R1A2B3010445; 2017R1A6A3A01005507)*

MO267

Investigations of artificial digestion of microplastic spiked with perfluorooctane sulfonate (PFOS) and related toxic effects using pregnane X receptor (PXR) in vitro bioreporter system

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During the past few years, research on microplastic (MP) particles has vastly developed. Several studies have shown that MP may cause either no or only minor toxic effects, and in most of the cases the effects were due to the additives or environmental pollutants sorbed onto the plastics. However, little is known about the desorption of pollutants or additives from plastic during digestion and subsequent effects in fish. For different chemicals the modelled desorption potential is to be expected in a non-toxic dimension. Therefore, the capability of gut surfactants to desorb pollutants from MP still needs to be tested. PFOS is a persistent organic pollutant that is shown to cause several adverse effects. The aims of the present study are 1) to investigate the desorption of perfluorooctane sulfonate (PFOS) from MP under simulated digestive conditions; and 2) to evaluate possible toxic effects of the desorbed toxicants associated with the MP using pregnane X receptor (PXR) *in vitro* bioreporter test system. PFOS was spiked on polyethylene particles (11 – 13 μm, 0.96 g/cm^3) in concentrations of 70 μg per gram of plastic. The prepared MPs were exposed for 4 days in artificial digestion fluid composed of sea salt (35 ‰) and 15 mM of the bile salt sodium taurocholate (NaTC). This solution is used to mimic the intestinal juice of the Atlantic Cod (*Gadus morhua*). NaTC is the main driver in solubilisation of nutrients during digestion in the posterior intestine since it has surface active properties. The simulated digestion over 4 days represents the average gut retention time of food in Atlantic Cod. After exposure, fluids will be analysed for PFOS using LC–MS/MS in order to quantify desorption. The same fluids will be tested using PXR *in vitro* bioreporter systems to evaluate the adverse effects from desorbed PFOS. Preliminary results reveal that less than 10 % of the initial PFOS concentration desorb from the MP particles. However, we expect a cellular response in the bioreporter test system since PFOS is known to cause effects in comparable low concentration ranges. The overall goal of this investigation aims for better understanding of desorption of pollutants from MP under digestive conditions which may also contribute to a more comprehensive environmental risk assessment of MP pollution.

MO268

Assessment of microplastics as a source and carriers of pesticides to earthworms (Eisenia fetida)

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Studies addressing the contamination of soils with microplastics (MP) are few and the impacts on soil biota are poorly known. Less than 10% of peer-reviewed publications covering the period between 1972 and 2019 addressed studies related to microplastics and terrestrial environments. Some questions can arise related with this significant environmental issue, and its impacts on soils and terrestrial ecosystems, mainly whether MPs could transport contaminants (e.g. agrochemicals) on the soil matrix and if they could be carriers of pesticides to soil organisms through ingestion. In order to contribute to the understanding of these issues, earthworms (*Eisenia fetida)* were exposed for 14 days to OECD soils containing MPs with two different sizes [large MPs (5 mm) and small microplastics (0.250 -1 mm)], previously sprayed or not with chlorpyrifos following the dose recommended by the supplier (4 L ha^{-1}). This agrochemical is a broad-spectrum organophosphorothionate pesticide with several agricultural applications to a variety of food and feed crops, and it is used worldwide. Acetylcholinesterase activity and thiobarbituric acid reactive substances (TBARs) were measured to track the exposure of earthworms to the MPs alone or combined with chlorpyriphos. The behaviour of earthworms in the test containers was also assessed, as well as the movement of MPs on the soil. The concentration of chlorpyrifos in soil after 14 days differed between treatments with MPs of different sizes [17.9 ng g^{-1} and 2442 ng g^{-1} for large (5mm) and small MPs (1 mm), respectively]. Despite the ability of the MPs to release chlorpyrifos to the soil, the earthworms have firmly avoided the 1mm MPs treatment. At the lowest concentration of chlorpyrifos provided by large MPs, earthworms avoided it, but the contact time with contaminated soil was higher, as shown by the enhanced level of TBARs and acetylcholinesterase inhibition.

MO269

Ecotoxicology testing of microplastics contaminated sediment by oligochaete worm Lumbriculus variegatus.

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Microplastics have been found in all aquatic ecosystems and environment. While impact of microplastics in marine environment has been widely covered, little is known about the risk in freshwater ecosystem. It is particularly relevant in the case of freshwater benthic organisms which apparently have a higher risk of exposure as a result of sinking microplastics onto sediments. Polyethylene microplastics (MPs) with a 10 µm particle size were spiked into sediment or water in three experiments. Survival, feeding activity, growth and reproduction of worms were used as end-points to evaluate the toxicity of MPs. First, worms were exposed to microplastics in different exposure routes water column, pore-water, and whole-sediment to assess the most harmful pathway. Second, different MPs concentrations ranging from 0 to 50 g/kg of MPs were set to analyse how the established endpoints may be altered with the increasing MPs concentration. Third, a layer of 1 mm MPs were placed at different depths, starting at 1, 4 and 7 cm, to study bioturbation effects of *Lumbriculus variegatus*. Feeding activity and growth was lower in whole-sediment exposure being these differences statistically relevant. Moreover, feeding activity has shown a clear dose-response correlation with increasing MPs concentration, in fact, at the highest concentration was almost zero. Growth showed remarkable decrease in higher concentration compared to controls. At 1 cm deep there was a slight mixing of the microplastics toward the upper layers due to the worms' movement. The worms reached the microplastics located 4 cm deep, however, the worms did not penetrate below that layer and never reached MPs at 7 cm deep. *Lumbriculus variegatus* did not feed on MPs present in the water, only feeding on MPs contained in the ingested sediment, being the main pathway whole-sediment exposure. This study has demonstrated the ability of *Lumbriculus variegatus* to mix MPs in the upper layer. The worm is able to mix MPs in the upper layer very close to sediment surface, but cannot burrow further than 4 cm deep. Indeed, it has shown very low MPs transport capacity. The obtained EC_{50} value (3.29 g/kg) for feeding activity suggests there is not an immediate risk for the worm. However, microplastics concentration are expected to increase, therefore, *Lumbriculus variegatus* and other more sensitive species may be in danger in the near future.

MO270

Effects of microplastic beads and their associated microbial communities on the sea urchin Paracentrotus lividus

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Microplastics (< 5 mm) are a global threat to the marine environment and they are attracting attention of researchers based on the consideration that, despite their documented widespread occurrence, little is known on their effects on the marine biome. In the sea, these particles undergo deep transformations by physical, chemical and biological factors. They also harbour microbial communities which contribute to their transformation and represent the so-called “plastisphere”. In this work we have investigated the uptake of virgin microplastics (fluorescent polystyrene beads) by the sea urchin *Paracentrotus lividus*. Uptake was observed both in the digestive and water vascular systems and was dependent on the size and concentration of the particles. Moreover, fluorescent beads were detected in the coelomic fluid and in the gonads. The number and phenotype of the immune cells, the sentinels of the environmental stress response, was characterized. Markers of oxidative stress, including ROS and RNS determination, enzymatic activities, antioxidant response were examined in immune cells and in tissues containing microplastics. Natural microbial communities adhered on microplastic fragments have been assessed in the Gulf of Naples during several surveys. Scanning electron microscope analyses of fragments collected by manta net show different degrees of colonization and dominant microbes are bacteria and diatoms. Based on the observation that in nature microplastics are always colonized by microbes, we have exposed the sea urchins to beads incubated in natural seawater from different coastal sites for different times to test the hypothesis that microbial communities modulate the effect on the sea urchin in terms of stress response.

MO271

µFT-IR imaging to analyze the effect of antifouling paint leachates on a freshwater microalga

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We evaluated the effect of the leachate of a mixture of antifouling paints on the growth and physiology of a freshwater microalga (*P. subcapitata*). We applied Fourier Transform Infrared micro imaging spectroscopy (μFT-IR) with a focal plane array (FPA) to examine the related metabolic changes of the organisms. The technique allows examining numerous algae cells individually, therefore provides a detailed view of the effects of environmental stressors. A mixture of antifouling paint particles (20-500 μm) was leached in algae growth medium at 20°C for 72 hours with liquid to solid ratio of 100. A dilution series of 4 with a factor 10 was prepared from the concentrated leachate. A growth inhibition test was performed with modifications according to *Water quality - Freshwater algal growth inhibition test with unicellular green algae, ISO Standard 8692, 2004*. Algae cells were subsequently deposited on a CaF_2 transmission window for μFT-IR imaging. A fraction of the window was scanned to create a mosaic with 1.1 µm pixel resolution on the FPA. The obtained spectra were processed applying baseline correction, standard normal variate correction, and smoothing using the Savitzky-Golay algorithm. The second derivative of the processed spectra was used in the PCA analysis. There was a significant difference in the growth rate among the samples (ANOVA, p=3.82?10^{-9}). The following Tukey HSD-test revealed that the growth inhibition observed in the concentrated leachate and the first dilution was significantly different from the control, while the growth rate of algae incubated in the more diluted leachates was not significantly different from the control. \n PCA analysis with the obtained spectra showed a slight grouping of the spectra by the degree of dilution of the leachate with extensive overlap among the groups. Particularly, spectra of algae exposed to the second dilution (100x) seemed to separate from the spectra of control and other dilutions. The PCA analysis suggests that exposure to the diluted leachates could have caused metabolic changes in some of the algae cells, while it did not affect the growth rate of the population.

MO272

Representative behaviour of nanoplastics in a salinity gradient: a micro-chip to go one step further?

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Plastic pollution is a global concern as plastic debris are present all around the world and especially in aquatic compartment. Up to now, it is well known that meso-plastics break down into micro and nanoparticles according to physical, physico-chemical and biological processes in aquatic environments. Evaluating nanoplastics toxicity in marine waters is a challenge considering their tendency to aggregate in presence of high ionic strength. Up to our knowledge, relatively few studies have considered nanoplastics aggregation before exposing organisms, whereas fate and toxicity of nanoplastics depends on their aggregation behavior. The aim of this study is to evaluate and understand the behavior of **environmentally relevant nanoplastics** in different salted medium before exposition of organisms. More specifically, we develop unprecedented methods to representatively disperse nanoplastics into a **salinity gradient** by designing and using a **microfluidic device**. \n Micro-channel is filled by NaCl solution or artificial marine water in one inlet, and nanoplastics dispersion in the other. At the two outlets, evolution of size distribution, fractal dimension and stability is investigated using *in situ* dynamic light scattering, static light scattering, laser induced breakdown detection, asymmetrical flow field-flow fractionation and zeta-meter. Different aged nanoplastics (100 to 500 nm) were prepared from pristine polystyrene and polyethylene materials, and also from environmental plastic debris sampled on Caribbean littoral. Standard polystyrene nanospheres (200 nm) are also used as a reference for comparison with aged nanoplastics and literature data. \n Both type of particles aggregates in the centre of the microfluidic channel, at the maximum transition of the salinity gradient. While polystyrene nanosphere creates and homogenous deposit, aged nanoplastics forms scattered large aggregates. This difference can be explained by their polydispersity. The hydrodynamic conditions in the microchannel seem to induce an aggregation pathways governed by Reaction-Limited-Cluster-Aggregation (RLCA) mode for the two types of particles. To the contrary, in the classical batch approach i.e. dropwise addition of NaCl solution into the nanoparticles dispersion and manually stirred, aged nanoplastics and polystyrene nanospheres present a characteristic aggregation pathway of Diffusion-Limited-Cluster-Aggregation (DLCA) mode.

MO273

Screening Method for Microplastics in Beach Samples

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Plastic, especially microplastic contaminate the environment all over the world. To avoid further entry, sources have to be identified. For this, methods for the extraction of microplastics from different matrices, in this case from sediments, have to be developed. Here, a newly developed flotation method for fast extraction and screening of microplastic particles (> 100 µm) in large sediment samples is described. Recovery experiments were made with different polymer particles (PE, PP, PS, EPS, PET, PU, PA and PVC). The method is based on the principle of flotation and allowed even separating microplastic particles with densities in the range of the separation fluid (PET, PVC) with excellent recovery rates (> 93%). A reverse airflow is included to support separation of the lighter microplastic particles from the denser matrix components. To minimize the amount of fine sand particles which clogged the filter and aggravated visual detection of coloured polymer particles in the suspension, pre-treatment of the samples proved necessary. Fine sand particles were removed by sieving through a 63 µm standard stainless-steel sieve for soil samples which also removes small microplastic particles (< 63 µm) from the sample. Recovery rates of particles in the size range 400 µm – 1000 µm increased to 95% with dry sieving and more than 97% with wet sieving. All in all, this method allows fast and efficient screening of microplastic particles (> 63 µm) in large beach sediment samples.

MO274

Developing new methodologies for microplastic extraction from lake sediments to understand the extent of contamination in UK lakes.

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Microplastics (< 5 mm) are persistent environmental pollutants characterised by heterogeneous physico-chemical properties and a broad range of shapes, sizes, colours and composition. Microplastics may be directly released into the environment at this size (i.e. pellets and cosmetic microbeads) when they are known as primary microplastics. However, the majority of microplastics are secondary, i.e. they originate from the degradation of larger plastic items. An important source of secondary microplastics is represented by fibres released during washing of synthetic garments. Although microplastic contamination is thought to be ubiquitous in aquatic ecosystems, very little is known about the scale and extent of contamination in rivers and lakes. In particular lake sediments, may represent an important sink for microplastics as well as providing a means to assess historical trends. To assess microplastic abundance and distribution in the sediments of UK urban and rural lakes, reliable methodologies for microplastic sampling and extraction from sediments are still needed. To develop such a methodology to separate microplastics from sediment particles, extraction experiments with reference materials have been conducted. Natural sediment has been spiked with known concentrations of fibres and microbeads, encompassing plastic polymers commonly identified in environmental samples. Plastic-spiked sediment samples have been added to a range of high-density solutions (NaCl,

$ZnCl_2$, NaI and sodium polytungstate) to evaluate the separation efficiency of different floatation methods. ---- The results from these experiments have helped develop a reliable method for microplastic extraction from lake sediments which can then be applied to determine spatial and temporal distributions of microplastics in UK urban and rural lakes. This study presents the results from the extraction experiments and the preliminary results about temporal and spatial distribution of microplastics in sediments from Hampstead Heath ponds in London (urban sites) and from the Norfolk Broads National Park (rural sites).
Keywords: *Microplastics, lake sediments, microplastic extraction, density separation*

MO275

Identification and Quantification of Microfibers and Microparticles via FTIR imaging and Automated Analysis

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The ubiquitous presence of small plastic particles and fibers (< 5 mm) with in the aquatic environment is of growing concern for sciences and societies. Nowadays these types of litter have already been found in the remotes areas of the world including the Arctic and Anarctic regions. Still, the analysis of these materials is a challenging topic as several steps are necessary like sampling, work up, and finally chemical identification. As most of these particles are barely visible by the naked eye, each of these steps has its own challenges. By FTIR imaging a rapid identification is possible and it was further improved via the automated analysis of complete filters. While the overall process is a powerful tool to characterize microplastic particles, the identification of microfibers was not possible within previous studies. To overcome this limitation we present a novel approach to measure, characterize and quantify microfibers and -particles concentrated onto membrane filters within one measurement. FTIR imaging was extended to fibers via a specialized sample handling to fix the fibers in the focal plane of the instrument. Further, after the automated analysis, an analytical procedure via Image Analysis was developed. Potential fibers were preselected from the dataset and further investigated. Different parameters allowed a detailed investigation with regard to object shape to differentiate small particles from short fibers. Afterwards, the fibers are investigated for each individual polymer type, their length and abundances. Via a filament closing process small gaps within larger fibers are closed to exclude an overestimation by overlaying objects. Subsequently the procedure was applied on datasets from studies of treated waste water to investigated type and number of micro-fibers and –particles within the effluent of the treatment plans. As first result in all of the investigated samples cellulose fibers were found. Moreover, synthetic fibers of different polymer types were successfully identified and consisted mainly of polyethylene, polypropylene, polyacrylates and polyamide. With this dataset we could show that we developed a versatile and easily applicable tool for the investigation of micro litter. The automated analysis of microfibers and –particles within one measurement further improved the necessary harmonization of microplastic research to lead to the implementation of standardized operational protocols (SOP) in the future.

MO276

Microplastic content in wastewater - a new thermoanalytical method

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The underlying Balance Method was originally developed for the determination of the fossil content in refuse-derived fuel. Recent activities at the TU Wien lead to an extension of the method to the field of (large) microplastics analytics. The concept is based on the distinctly different macro elemental composition of moisture and ash-free biogenic and fossil organic matter. Determining the content of the elements C, H, N, S and O in the dried sample and in the ignition residue is sufficient for calculating the corresponding plastic content (= fossil content): *Xb * mb + Xf * mf = (TXsample -TXi * mi)/(1-mi)* (Eq. 1) The mathematical fundament of the Balance Method is a system of equations, formulated according to the balance written above (Eq. 1). Whereby each element (= C, H, N, S, O) represents one equation in the system. The elemental composition of the sample and corresponding ash *(*TXsample, TXi) together with the ash content of the sample (mi) are measurable values. The material data constants of biogenic and fossil matter are defined as the elemental composition of the pure biogenic (Xb) and pure fossil (Xf) fraction of a certain sample type. A data reconciliation algorithm is applied to calculate the quantity of the unknown mass fractions (biogenic mb, fossil mf) including associated uncertainties. Several test series with different types of wastewater as well as environmental samples have already been analyzed. Complementary recovery tests emphasized the applicability of the Balance Method for microplastics quantification. However, specifying the material constants Xb and Xf demands a profound understanding of the material composition of a certain sample type. Preliminary sample information is crucial and can be provided by complementary methods, capable of polymer identification like Py/TED GC-MS or FTIR/ATR IR or Raman spectroscopy. Once the constants are fixed, the effort in the laboratory is reduced to a three-step analyzation procedure. Step 1 is sample preparation and homogenization including chemical oxidation (if necessary). Step 2 is the determination of the ash content by a simple loss on ignition test. The last step is strictly machine dependent and comprises the elemental analysis of the sample and the corresponding ash. This straight forward procedure represents a cost-efficient option in the field of microplastics quantification, especially for monitoring approaches.

MO277

Combining sampling of liquid matrices and an automated sample preparation for microplastic analysis using FPA µFTIR Imaging

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Microplastic (MP) pollution is a global issue affecting almost all types of matrices such as marine waters, freshwaters, wastewaters, etc. In order to quantify the amount of microplastic in a sample, unwanted organic matter has to be removed. This is done by procedures including wet peroxide oxidation and enzymatic digestion. Typically the sample preparation is done in open laboratory vessels, involving the transfer of samples between vessels and filtering between steps. This procedure does though leave the sample open to the surrounding atmosphere, which increases the risk of contaminating it by airborne microplastics. Another issue is that the procedure is labor-intensive and prone to human mistakes. This study proposes a methodology to minimize sample contamination by performing sampling and sample preparation in the same closed device, first opening it when the microplastics concentrate is ready for final analysis. It does so in an automated way, thereby reducing the labor needed to conduct the sample preparation. The study furthermore present data on microplastics contamination rates and recovery rates applying this approach. The device is a custom-made filter holder that can hold a steel filter with a diameter of 150 mm and a mesh of for example 10 μm. All parts are made of stainless steel and can be muffled. The cleaned and closed filter unit is taken to the field and a sample collected by a vacuum pump sucking the sample through the filter or by using a pressure pump without plastic parts in contact with the water. Back in the laboratory, the device is placed in a rag and slowly flushed with a sequence of filtered (2 μm) treatment solutions: SDS, cellulase, protease, hydrogen peroxide. Between the steps, the filter is flushed with Milli-Q water to ensure proper separation of the chemicals. The process is automatized by a PLC-controller in order to minimize the amount of labor put into the sample preparation. Upon treatment, the filters are extracted inside a laminar flow cabinet and the particles concentrated. Finally, a sub-sample of the concentrate is analyzed by FPA μFTIR imaging.

MO278

Application of novel large area ATR (LAATR) for use with µFTIR Imaging technology: expanding analytical options for measuring <10 µm microplastic particles, fibres and surfaces.

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With research on microplastics increasing every year, a consensus is forming on appropriate analytical methods. With light microscopy no longer being considered as a truly acceptable standalone analytical method for identification of smaller size ranges of microplastic, vibrational spectroscopy methods have become a staple holder in methods considered for standardisation of analytical methods. With the rise in interest for measuring microplastic particles smaller than 100 μm or even less than 10 μm, expansion of analytical methods using Focal Plane Array (FPA) based μFTIR imaging are becoming increasingly of interest. A novel ATR method has been made available called the large area ATR (LAATR), which consists of a diamond covered zinc selenide ATR unit. It is theorised to be especially good for analysis of particles and fibres under 10 μm in size. Furthermore, it has large potential for analysing surface characteristics of particles with spatial accuracy of only few microns. This study aims to provide clear proof of concept considering its function and performance, showing why it should be considered for a standardised mode of analysis when identifying and quantifying microplastics in the 1-10 μm size range.

MO279

Non-target analysis to measure microplastics and emerging debris by automatic microFTIR with Multivariate Curve Resolution analysis and Correlation analysis

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General analysis method to measure microplastics are FTIR analysis after picking potential MPs on a membrane, followed by H2O2 digestion and density separation processes. But fine MPs whose sizes are less than 100 µm can not be picked up. Transparent small particles are also picked up. The picking process and FTIR analysis need much time and hard work. FTIR analysis is also difficult to classify MPs to polymer groups because there is difference between standard polymer spectra in libraries and those from environmental samples. New standard analysis methods are needed to reveal the fine MPs contamination and to compare research results qualitatively and quantitatively in the world. This present proposes a new analysis method with automatic microFTIR with Multivariate Curve Resolution analysis and Correlation analysis. The microFTIR can measure IR spectra of all debris including MPs on the membrane automatically. The main spectra can be extracted from the IR spectra data by Multivariate Curve Resolution analysis (MCR). These results can reveal quality of major debris. MPs sometimes are classified to minor debris. Therefore, correlation analysis can be identify MPs by comparison the spectra to library data which is composed of spectra of environmental samples. After these analysis procedures, image mapping data for each polymer can be performed. Sizes and numbers of all polymers and other debris can be also counted automatically. This novel analysis method was applied to domestic wastewater, oyster and river water. By this method, new polymers and new emerging debris which the general analysis can not detect were found. This method can also detect more fine MPs very easily than we expected.

MO280

Misidentification of PVC microplastics in environmentally weathered samples

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Contamination of the marine environment is of increasing concern. Microplastics (MPs) can be present in the environment as manufactured microplastics (known as primary microplastics) or as a result of the continuous weathering of plastic litter, which yields progressively smaller plastic fragments (known as secondary microplastics). Once released, plastics and microplastics (MPs) may be subjected to degradation by several agents or routes, such as solar radiation, mechanical forces, and microbial action. Weathered plastics collected from the marine environment show considerable physical and chemical differences to pristine plastics [1]. Therefore, the assessment of the changes and processes that MPs suffer during weathering is essential to understand their effects in the environment and to their correct identification. PVC is the least stable of the high tonnage polymers as it has the highest sensitivity towards UV radiation and, therefore, photo-degradation is of highest importance. When PVC is exposed to sunlight, dechlorination is the first step, which leads to the formation of conjugated double bonds in a polyene polymer and hydrochloric acid [2]. Although thermal and UV stabilizers are added to extend the lifetime and stability of PVC products that are exposed to sunlight, MPs that remained for a long time in marine environments will be highly degraded. Moreover, existing methods for the assay of microplastic fragments typically involve either a flotation or density separation step using dense salt solutions. A saturated sodium chloride (density: 1.2 g cm^{-3}) is a common choice to density separation of MPs, but it could be insufficient to extract PVC microplastics, which have higher density (1.38 – 1.41 g cm^{-3}) [3]. The combination of all these factors, could make PVC MPs to be underestimated when analysed in environmental samples, making the direct identification of PVC an analytical challenge. In this work, chemical and physical changes on a standard PVC MP are characterized by ATR-FTIR, SEM and X-Ray Fluorescence analysis. [1] Silva, A.B. *et al.* 2018. Anal Chim Acta 1017: 1-19 [2] Gewert, B. *et al.* 2015. *Environ. Sci.: Processes Impacts* 17(9): 1513-1521 [3] Technical report. 2018. DOI:10.13140/RG.2.2.36256.89601/1 **Acknowledgements**: This work has been supported by Xunta de Galicia partially financed by ERDF (ref ED431C 2017/28); Ministry of Economy and Competitiveness PCIN-2015-170-C02-01-BASEMAN project (JPI Oceans), CTM2016-77945-C3-3-R

MO281

3-dimensional imaging of nanoplastic transport through a sand column using magnetic resonance imaging

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Comparatively little is known about the fate and impact of micro and nanoplastics in soils. Research in this field is complicated by the opaque nature of soil and the abundance of organic matter which is difficult to distinguish from plastics. Testing and establishing suitable methods for measuring micro- and nanoplastic presence and mobility in intact soils is at the forefront of ongoing research. In this pilot study we were explored the potential of quantitative 3-dimensional magnetic resonance imaging (MRI) of nano-plastic transport through soil like material, using nano-particles with superparamagnetic properties. The applied nano-particles were 40 nm diameter dextran spheres, coating an 8 nm diameter iron oxide core (Fe_3O_4). Particles were terminated with carboxyl groups and were doped with a fluorescent material (rhodamine b). A pulse of a nano-plastics suspension containing 0.5 mmol l^{-1} iron was applied onto a packed sand column (3 cm diameter, 6.3 cm height) under near-saturated steady state water flow (1.2 ml min^{-1}) while the sand column was placed inside the high-field MRI scanner at the IBG-3 in Jülich. Eleven consecutive 3-dimensional MRI images were obtained with a resolution of approximately 0.39 x 2.2 x 0.79 mm in x, y and z directions using a stop-flow approach (the water flow was stopped during MRI image acquisition to suppress nano-particle transport). The MRI experiment was preceded by consecutive additional leaching experiments on the same column at the same flow rate but without flow interruption. Here we used i) chloride ions as a conservative tracer; ii) fluorescent polystyrene nano-particles of the same diameter and similar surface termination as the dextran particles; iii) the superparamagnetic dextran particles to investigate possible transport differences caused by the flow interruptions during MRI scanning. The corresponding three breakthrough curves were analyzed in the effluent, exploiting electric conductivity contrasts or fluorescent properties. First results show that MRI images provided excellent means to separate the dextran particle plume inside the column from the background MRI signals. Preliminary evaluations of the effluent breakthrough curves indicate very similar transport properties for all investigated substances. We infer that the dextran particles with the iron cores may be suited as an MRI detectable proxy for polystyrene plastic transport and mobility in intact soils.

MO282

Fingerprinting of chemicals released from plastic polymers by UV-light

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Plastic production has been increasing over the last decades and has led to pollution of both the marine and terrestrial environment by (micro)plastic. The need for good analytical methods to determine the composition and concentrations of plastic in environmental samples is growing. Currently detection of plastics is done mostly by FTIR or Raman spectroscopy, and sometimes by pyrolysis GC-MS. These methods depend on an extensive sample preparation and may be specific for certain size fractions of plastic. Here we propose a new detection strategy which theoretically does not depend on any sample preparation and could potentially be applied to macro-, micro- and nanoplastic detection. The goal of this study was to establish fingerprints of the mixture of chemicals released by different plastic polymers under UV-light to identify plastics in unknown samples. We developed a "weathering wheel" in which plastic particles in the presence of water and sand were exposed to UV-light. We exposed cryo-milled PE, PP, PS, PET, PU and PVC particles (0.4 μm – 1 mm) for four days. The chemicals released into the water were subsequently concentrated using solid phase extraction (Oasis HLB Plus, Waters) and analysed by liquid chromatography coupled to high resolution mass spectrometry (LC-Orbitrap Q Exactive HF, Thermo). By using non-target screening the full spectrum of detected peaks was used to establish a chemical "fingerprint" for each polymer. At least 100 characteristic peaks for each polymer were detected. We then examine the hypothesis that these fingerprints can be used to identify the presence and composition of mixtures of plastic polymers in unknown samples.

MO283

How circular are plastic flows today, and how circular can they be tomorrow? - A material flow analysis for selected plastics in Switzerland

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Recycling of plastics can reduce the need for natural resources and energy. Currently, no good overview about the flows of secondary plastics into new products in Switzerland exists. To fill that gap, and with the further aim to propose suitable plastic recycling strategies, a static material flow analysis (MFA) for selected plastic types in Switzerland has been conducted. The focus was on the connection of the end-of-life treatment with product manufacturing. Therefore, the flows to and from different processes of the recycling chain, starting from the waste collection, have been investigated in detail. The plastic products have been grouped into categories with similar characteristics and requirements. The amount of secondary material produced and used within different product categories has been determined. It has been found that Polyethylene Terephthalate (PET), High-Density Polyethylene (HDPE) and Low-Density Polyethylene (LDPE) constituted the largest share of the secondary plastics produced in mechanical recycling in 2017. The recyclate originated mainly from PET and HDPE consumer bottles and from LDPE non-consumer packaging films. Typical applications for the secondary polymers are consumer bottles, tubes and pipes. Currently, the use of secondary polymers is often limited by (unknown) material quality. Contaminants from previous applications as well as additives from polymer manufacturing may hamper closed material cycles. In order to investigate possible future scenarios, the static MFA will be transformed into a dynamic MFA considering individual product lifetimes. The results of the analyzes can serve as a basis for further investigations, including life cycle and risk assessments. This finally allows to determine an optimum degree of recycling and provide a basis for regulatory decisions.

MO284

Chemical transfer from food contact material - A statistical model with limited data requirement and increased predictive power

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Plastic packaging is used to improve the shelf life, and to help ensure quality and safety of food products. Plastic packaging materials, called food contact materials (FCM), can be a potential source of chemical contaminants in food products. The amount of chemical (hereafter migrant) transferred from the packaging material to food can be determined experimentally or using migration models. Process-based migration models have a limited applicability domain and their predictive performance is low if only estimated chemical-specific diffusion coefficients are available. To overcome these two limitations, we aimed to develop a statistical model with higher accuracy and predictive performance. We used linear mixed effect models (LMM) and the database of the US Environmental Protection Agency (N=10,235 migration results, for 70 migrants, 16 packaging types, and 117 food/food simulants) to predict the fraction of migrant transferred from the FCM into the food. The model statistically relates the fraction transferred to factors related to the migrant, food product composition, FCM, and experimental design. The predictive performance of the LMM for transfer fractions of new migrants for FCM and foods that were part of the database was clearly higher than the process-based model when only estimated diffusion coefficients were available (Coefficient of Efficiency (CoE) of 0.46 for the LMM vs. -0.002 for the process-based model). The predictive performance of the LMM was also higher for new FCM with migrant and food combinations present in the database (CoE = 0.50). Our statistical model confirmed the negative relationship between the fraction transferred and the molecular weight (MW) of a migrant and the positive relationship between the fat content of the food and the fraction transferred. The relationship between the MW and fraction transferred was also influenced by the FCM's crystallinity: large chemicals (MW>400 g/mol) migrated more from FCM with low crystallinity than from FCM with high crystallinity. Our results indicate that statistical modelling approaches can help improve the accuracy of migration predictions, while keeping the complexity and input data requirements to a minimum. This method could make assessing chemical exposure from FCM easier in high-throughput risk screening exercises or life cycle assessment-based approaches as long as the starting concentration of the migrant in the FCM is known.

MO285

Mechanical stress causes microplastic release from PET mineral water bottles

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Plastic particle ingestion has become of concern as a possible threat to human health. Previous works have already explored the presence of microplastic (MP) in the bottled drinking water as a source of MP intake. Here, we consider the release of MP particles from single-use PET mineral water bottles upon exposure to mechanical stress utilizing scanning electron microscopy (SEM), which allows the implementation of morphological and elemental analysis of the plastic material surface and quantification of MP concentrations in sample water. The aim of this study is to better evaluate the sources of MP intake from plastic bottles, especially considering the effect of daily use on these bottles such as the abrasion of the PET and PE material. For that, we analysed MP release of PET-bottlenecks and PE-caps on their surfaces after a series of bottle openings/closings (one time, ten times, 100 times). Furthermore, we will investigate the occurrence of MP particles on the inner wall surface of the PET bottles and measure MP concentrations of the water after exposing the bottles to mechanical stress (squeezing treatment; none, one minute, ten minutes). All investigated bottles were purchased in Italian supermarkets and included different brands and water types. An in-house manufactured filtration apparatus was used to filter the sample water. To assess sample contamination or particle loss during the procedure in the lab, blank and positive control samples were included. The first results show a considerable increase of MP particles occurrence on the surface of PET and PE material (bottleneck and caps) after opening and closing the bottles 100 times. Considering these findings, MP concentrations in the bottled mineral water are expected to increase after exposure to mechanical stress (squeezing treatment). More information about MP concentration in mineral water - possibly increased through release of particles from the bottle inner wall - will be presented in the conference. In addition to the implications of the results, also the applicability of the used filtration and analysis technique will be discussed.

MO286

On the pathway towards the standardization for exposure assessment throughout life cycle of nanocomposites

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Both researchers and legislators have claimed the necessity to standardize the exposure assessment throughout the life cycle of polymer nanocomposites. In the present study we have developed and compared three different operational protocols to investigate changes in particle emission behavior of mechanically degraded polypropylene (PP) samples with different fillers including talc and not reinforced PP (as reference samples) and two types of nanoclays: wollastonite and montmorillonite. The presence or absence of engineered nanomaterials (ENMs) in the nanocomposite formulation has been investigated as the main factor causing differences in the particle emission profile. The three protocols, despite the fact of being carried out using the same samples and operating conditions, have yielded different figures for particles generation. Here we propose an integrative approach providing released particles as a function of the quantity of removed material in a certain time. The proposed option would allow comparing variations in the number of emitted particles by means of different mechanical processes. Finally, we have also concluded that no universal conclusions can be derived on how nanofillers modify particle size distribution and particle concentration of released particles during mechanical degradation of solid nanocomposites as it is a multidimensional phenomenon. Acknowledgements: LIFE SIRENA - GAN LIFE11 ENV/ES/596

MO287

Fate of Particulate Plastic in Sewage Sludge Treatment and Transport Behaviour in Unsaturated Porous Media

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While the occurrence of particulate plastic in the environment is widely discussed, mechanistic understanding of particle fate and transport is limited as few process oriented studies exist. Research on this topic is particularly limited due difficulties to detect plastic in complex matrices. To overcome these challenges, we synthesized metal-doped microplastic fibers and nanoplastic particles and successfully used them to trace particulate plastic in complex systems by measuring the metal as a proxy for plastic. In particular, in a pilot-scale wastewater treatment plant (WWTP), we found that >95% of the plastic spiked into the system was removed from the water column via the sewage sludge. Since 50% of sewage sludge is used as agricultural fertiliser in Europe, this may play an important role for plastic accumulation on agricultural fields. Still, a detailed assessment of mass flows, especially leaching from the sludge and further transport within the soil column, is missing. To help close this knowledge gap, we continued to investigate the transport of metal doped plastic particles from the sludge by-product of our previous experiments. A lifelike association of the plastic with the sludge matrix can be assumed, since the plastic underwent a realistic waste water treatment process. Sludge treatment consisted of anaerobic digestion followed by effluent dewatering by a decanter centrifuge. The mass balance over the dewatering process showed that the largest share of the plastic stayed associated with the solid fraction and only a small portion was recycled to the WWTP influent via the supernatant. From this, it was concluded that the major share of plastic particles are spread on agricultural fields in sludge application as fertiliser. Transport and leaching experiments were subsequently conducted with the processed sewage sludge. To reproduce different sludge application practices, samples with different water contents were tested. Transport studies in unsaturated glass bead columns were conducted both with pristine plastic particles as well as with plastic containing sludge. To decouple the process of plastic detachment from the sludge matrix and the migration through the column, leaching tests in the similar setting were also conducted. By combining the results from both experiments, first insights into the transport behaviour of microplastic fibres and nanoplastic particles were gained that can help to better understand the fate of plastic in soils.

MO288

Optical roughness - A suitable parameter to describe the fragmentation of plastics?

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Plastics are important products with a wide range of applications in industry and the household sector because they are inexpensive and durable. Unfortunately, these advantages lead to miss use and mismanagement and large quantities of plastic end up uncontrolled in the environment. Littering is hereby an important path of entry. After their introduction into the environmental, a number of factors (e.g., UV radiation, mechanical stress, and biodegradation) lead to the fragmentation of larger plastics parts into smaller plastic pieces and finally into secondary microplastics (pieces < 5mm). Within the scope of the BMBF (Federal Ministry of Education and Research) project ENSURE (Development of new plastics for a clean environment by determining relevant entry paths), the fragmentation process in semi terrestrial environments will be investigated. For this purpose, several shore zones with wave impact are set up in mesocosms of the stream and pond simulation system (FSA) of the Federal Environment Agency (UBA). Various plastic test specimens of different shapes are added in the artificial shore zones. The fragmentation processes of macroplastics and rates of microplastic formation in the environment is crucial and not sufficient investigated by now. In order to investigate these processes, suitable methods and parameters have to be found first. One suitable parameter could be the change of the surface roughness of the test specimens. This contribution presents the investigation to use optical roughness measurements for the fragmentation process of plastics and compares the results of the initial roughness measurements with gravimetric measurements of macroplastics in mesocosm.

MO289

Drinking plastics?

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The presence of microplastics has been previously reported in numerous natural habitats as well as in the digestive tracts and cell tissues of a number of species. Recently, microplastics were also detected in food and drinking water designated for human consumption. Consequently, researchers all over the globe remark concerns about the potential implications for human health. Beside incomplete removal during water purification, the deterioration of plastic equipment and pipes used during water purification or transport in waterworks is likely a source for microplastics in drinking water. Up to date, limited data is available on microplastics concentrations in drinking water. The few studies conducted so far reported about generally low numbers of microplastics in drinking water but also about contamination possibly due to handling and the use of plastic equipment during sampling and processing. Therefore, it is essential that quality assurance is in place and demonstrated, but also that an adequate volume of drinking water is assessed, in order to reliably analyse microplastics in drinking water distribution systems.We designed a drinking water sampling workflow to reduce and control the contamination risk during sampling and processing. Following, up to 1 m^3 of drinking water were sampled per site directly from the distribution pipes through 3 μm stainless steel filters in a closed steel filter system. Using μFT-IR imaging enabled us to assess the presence, identity and quantity of small microplastics (>10 µm).

MO290

Development of a standard addition method for the preparation of water samples for microplastic analysis

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It is well known, that huge amount of plastic waste enters the environment. Despite of their resistance for microbiological degradation, different physical-chemical processes (e.g. photooxidation) causes chain cleavages in the polymer structure and result in smaller and smaller fragments. This is the main reason for one of the most important problems of the ecosystem: the presence of microplastics in the environment. Microplastics (plastic prticles < 5mm) have been detected globally in a wide range of environmental elements, but the methods of sampling, sample preparation and analysis are not harmonised yet. Different methods apply different steps to clean up the microplastics as much as possible from the matrix of the sample (e.g. plankton in water or sand particles in sediment). For this, usually density separation, oxidation and filtration are applied. The number of particles may be decreased after the preparation if the equipment or solutions for these steps are not chosen well enough. Also, sample transfer from one step to another during the preparation may cause losses of particles. The efficiency of the extraction processes is barely monitored, the lack of these measurements causes uncertainty in the observed environmental concentration. To improve the knowledge on sample preparation efficiency, we developed a standard addition method, where recovery rates can be analysed fast and precisely. For the first tests three different equipment – the widely applied separation funnel and two specially designed glassware – have been used with three saturated salt solutions ($NaCl$; $CaCl_2$; $ZnCl_2$) that are also common in the literature. Commercial fluorescent microspheres with different densities and diameters have been added to the samples, tests have been conducted in three replicates. Results show that the glass flotation prototype with conical fittings provided the best results when it was applied with $ZnCl_2$. The prototype with the associated standard operating procedure has been also validated using field collected water samples, recovery rates have been detected over 90%. This work has been supported by project no. KFI_16-1-2017-0477, that has been implemented with the support provided by the National Research, Development and Innovation Fund of Hungary, financed under the "Vállalati KFI_16" funding scheme.

Developing Trophic Ecotoxicology to Support Impact Assessment across Ecosystem Boundaries (P)

MO291

Can amino acid-specific stable carbon and nitrogen analysis improve quantifying arctic food web dynamics of persistent organic pollutants and mercury: a case study on the Greenland Sea food web

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Evaluating *in situ* biomagnification is a valuable posterior approach to further assess the chemical bioaccumulative potential of a substance identified a potential contaminant by its physicochemical characteristics, such as the octanol-water partitioning coefficient. The investigation of trophic magnification factors (TMFs) has consequently become the quantitative backbone of assessing real-world food web dynamics of contaminants. The reliability of TMFs rests however upon the accuracy with which the relative trophic level of the individuals investigated for their contaminant load is determined. In this respect, the advent of measuring stable carbon and nitrogen isotopes of individual amino acids, rather than of bulk tissue, seems particularly promising in eliminating uncertainty in food web baseline stable isotope values necessary to reliably determine relative trophic levels. The isotopic signature of essential amino acids remains in fact relatively unaltered throughout the food chain and therefore provides the food web baseline value for which previously only approximations could be employed. The Greenland Sea is a pristine Arctic marine ecosystem though its food web has up to now never been investigated for exposure to contaminants, such as mercury, persistent organic pollutants and per- and polyfluoroalkyl substances, typically found in the Arctic despite its remoteness from primary sources and ongoing mitigation under the Stockholm and Minamata Conventions. Being particularly interested in evaluating the performance of the amino acid-specific analysis based TMFs we investigate the food web dynamics of the above substances using both bulk tissue and compound-specific analysis for stable carbon and nitrogen isotopes. The food web investigated here composes particulate organic matter (POM), two copepod species, four amphipod species, three euphasiid species, twelve fish species, and four seabird species. The availability of POM and different primary consumers allows us to uniquely present on their bulk tissue and compound-specific stable isotope values, most often not available in food web assessments, and as such compare the performance of the TMFs resulting from both methods. Moreover, POM and copepods were collected at several locations allowing us to evaluate the spatial variation in food web baseline isotopic values, and how adequately amino acid-specific analysis can resolve this frequently stumbled upon issue when employing bulk tissue analysis.

MO292

Can elevated radiocaesium in benthic fish off Fukushima be explained by trophic transfer through benthic food webs?

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Seven years after the accident at the Fukushima Dai-ichi Nuclear Power Plant (FDNPP), activity concentrations of radiocaesium (^{137}Cs and ^{134}Cs) still remain elevated in some bottom sediments, benthic invertebrates and benthic fish in the surrounding marine environment. The long ecological half-life of Cs isotopes in these organisms is thought to be partly due to delayed accumulation through the benthic food web, but the exact mechanisms are not fully understood. This study analysed in more detail the benthic food web structure at a number of sites at varying distances from the FDNPP and related this to the activity concentrations of Cs measured in the sediment, benthic invertebrates and benthic fish at each site. Food web structure was elucidated by visual analysis of fish stomach contents and stable isotope (SI) analysis (δC, δN) of benthic invertebrates and fish. Visual gut content analyses indicated that the fish ate predominantly small shrimps, gammarid crustaceans and a range of polychaete worms, with bivalve and gastropod molluscs also being taken. The stable isotope distribution in the fish and invertebrates, as analysed by fitting of mixing models, further strengthened the food web structure theorised by the results from the visual gut content. Concentrations of Cs in the sampled invertebrates varied by two orders of magnitude, with the highest values in infaunal benthic deposit feeders such as sea cucumbers, heart urchins and some polychaetes, sampled in soft sediments at 120m water depth. Cs concentrations were more strongly related to the functional group of the organisms than, for example, proximity to or direction from the

FNDPP. Fish Cs concentrations were also highest at offshore sites. Invertebrate SI signatures varied widely (δC: -7.8 to -22.6; δN: 6.3 to 13.3), but those of benthic fish did not (δC: -16.1 to -20.0; δN: 10.1 to 14.0). There was no obvious relationship between δC or δN and Cs concentrations. Benthic fish in this area are generalist feeders and their prey items contain varying amounts of radiocaesium. Sediments in the area near FDNPP still seem to be a source of radiocaesium to the food web, with the highest concentrations in the sediments also reflected in concentrations in benthic invertebrates and fish. However, there is considerable spatial variability in Cs concentrations in all compartments, as well as in food web structures, leading to unpredictability in Cs accumulation, which is a challenge for risk assessment.

MO293

Does pesticide contamination affect assemblages of algae, macroinvertebrates, and biofilm meiofauna in Swedish agricultural streams?

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We studied the effects of agricultural pesticides on assemblages of benthic macroinvertebrates, epibenthic algae, and biofilm meiofauna in 32 streams in landscapes with intensive agriculture in Southern Sweden. We used data from a pesticide screening study (montly of biweekly grab samples) and data from 4 national monitoring sites (time-integrated samples) that had been collected during the spraying season (i.e. April–October). We used acute EC50-values to calculate ∑TU as a measure of exposure to observed pesticide mixtures. These calculations showed that some 5% of the collected water samples had ∑TU that exceeded EU's uniform principles (i.e. ∑TU > 0.01 for *Daphnia*and fish). The vast majority (>88%) of ∑TU for algae was lower than 0.01. Only for 12 of 438 samples (or less than 3%) did ∑TU for algae exceed 0.02, of which 6 in the time-integrated samples collected in the monitoring sites. Benthic algal assemblages showed a large variability in species composition and biomass, but mostly resembled assemblages typical for nutrient-rich streams. The share of diatom frustule deformations exceeded 1% in 10 of 32 streams, suggesting that these sites could be impacted by pollution. Benthic macroinvertebrate fauna showed relatively high ASPT-scores in all but three streams, indicating high or good ecological status according to legal Swedish assessment criteria. However, when the SPEAR-pesticides metric and assessment criteria by Beketov et al. (2009, Environ. Poll. 157: 1841–) were applied, only four of the streams were judged at good status. Interestingly, ASPT and SPEAR-pesticides show a strong correlation (r=0.79), suggesting that both metrics largely quantify similar stressors, but that there is a mismatch for the assessment scale. Obviously, pesticide mixtures are one of several stressors in a complex stressor scenario that act on these streams. Analyses of biofilm nematodes using the Nema-SPEAR index showed that all but three streams were at high or good status. RDA showed that only meiofauna assemblages correlated significantly to ∑TU for *Daphnia*. This may be due to the fact that meiofauna assemblages in epilithic biofilms are more exposed and that pesticides may accumulate in biofilms.

MO294

Does the discharge of chemicals to the environment harm wildlife populations? Introducing the ChemPop Project

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We introduce a new project funded by NERC in the UK, which will address the research questions I: What are the impacts of hazardous chemicals on populations, ecosystems and ecosystem services and II: their relation to other pressures in the environment. We will address these by mining Britain's extensive wildlife monitoring databases which were instigated up to 40 years ago. Traditional chemical risk assessment relies on laboratory ecotoxicity studies, and then to model (in effect speculate) what the population or ecosystem functioning consequences might be. We aim to move beyond these current limitations by interrogating wildlife population data in the terrestrial, freshwater and marine spheres in the context of chemical exposure to help move the subject further forward. Our high-level aim is to identify which populations and environments are doing well under the current chemical regime and which are not. This will allow the UK to focus its research where the greatest wildlife declines are occurring. This should bring clarity to the issue of chemicals in the environment that continues to provoke great anxiety and uncertainty. Chemical exposures we will examine include pesticides in the terrestrial and freshwater environments, the de facto mixture in wastewater effluent, metals and persistent organic pollutants. We will be looking at macroinvertebrates and fishes in our rivers, invertebrates and sparrowhawks on land and cetaceans (dolphins and killer whales) off our coasts. These environments and species represent current concerns across the natural environment for both diffuse and point source pollution. We will focus on species and taxa that are either core providers of ecosystem services or represent aspects of native biodiversity identified by the public as important. There are many stressors and compensating factors other than chemicals that can influence wildlife populations. We will ask how important are chemical stressors in relation to other pressures in the environment? By comparing long-term and spatially explicit trends in natural populations, with the response predicted by classical ecotoxicity as reported in the literature, we will evaluate whether such tests are indicative of impacts in the wild. This is essential to assess to what extent traditional risk assessments, typical of those used in the Water Framework and similar Directives, are predictive of outcomes for wildlife populations in terrestrial, freshwater or marine environments.

MO296

Environmental behavior and bioaccumulation of contaminants of emerging concern in northern freshwater ecosystems

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Remote northern ecosystems are often viewed as net receptors of persistent organic pollutants (POPs). These contaminants are transported to Arctic regions via long-range atmospheric transport, but can also have local sources within the Arctic. While concentrations of some legacy POPs have declined in Arctic ecosystems, there is increasing concern about emerging organic contaminants that may have similar properties: toxicity, bioaccumulative, persistent and capable of long-range transport. However, our understanding of their environmental behavior and bioaccumulation in the Arctic environment and ecosystems is still limited. Hence, our objective is to investigate their concentrations, trophic transfer and bioaccumulation in Arctic freshwater ecosystems. We selected two lakes located in Northern Norway, Takvatnet (69 °N 19 °E) and Storvannet (70 °N 23 °E). Their ecosystems are well-studied, and they have similar food webs with Arctic Char (*Salvelinus Alpinus*) and Brown Trout (*Salmo Trutta*) as top predators. Takvatnet is a remote lake, with no known local sources of contaminants. Storvannet is situated in a populated area, with several local sources of organic contaminants. Sediments, zooplankton, benthos, three-spined sticklebacks (*Gasterosteus aculeatus*), char and trout were sampled from Takvatnet (2017, 2018) and Storvannet (2018). Passive air and water samplers were also deployed. Morphometric data, otoliths and samples for stable isotope analysis ($\delta^{15}N$, $\delta^{13}C$) and lipid content were collected from all organisms. Initial samples of muscle and liver from char and trout in Takvatnet were analyzed for selected chlorinated paraffins (CPs), Dechlorane Plus and analogues, new brominated flame retardants (NBFRs) and some legacy POPs including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and dichlorodiphenyltrichloroethane (DDT). Preliminary results show higher PCB concentrations in trout (muscle: 355 ng/g lw, liver: 187 ng/g lw) than in char (muscle: 101 ng/g lw, liver: 111 ng/g lw), consistent with trout occupying a higher trophic level. Dechlorane 603 and short- and medium chained CPs were detected near detection limits, while no NBFRs were detected. Contaminant levels throughout the trophic levels in both lakes will be used in combination with a mechanistic model to improve our understanding and predictive capability of environmental drivers, trophic transfer and bioaccumulation of these compounds in freshwater ecosystems.

MO297

Feeding composition, trophic level and location affects the Persistent Organic Pollutants and Emergent compounds levels in Andean Condor

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Persistent organic pollutants (POPs) are now known to pose a potential risk to human, wildlife and ecosystems health. Due to their persistence and volatility, they can travel long distances and settle in places far away from their origin. In Chile pollutants are distributed in an altitudinal, latitudinal and anthropogenic gradient, presenting higher concentrations in central Chile. Otherwise, due to their toxicity, they may generate varied health effects, having consequences in reproduction, early development and behavior in individuals and populations. The bioaccumulation and biomagnification capacity of POPs affects mainly the species located at the top of the food chain. The main entry route, at least in terrestrial organisms, is through ingestion. Therefore, feeding behaviour can influence the

exposure to pollutants and consequently their health status. The Andean condor (*Vultur gryphus*) is especially vulnerable to POPs because it is scavenger and very long-lived. According to our knowledge, studies on this subject in the Andean condor are gap to be filled until now. Therefore, the objective of this study is to evaluate the relationship between food sources and contaminant levels in two high density Andean condor nuclei in Chile. Identify the composition and trophic level of food resources through analysis of pellets and stable isotopes of Carbon (C) and Nitrogen (N) and finally relate the levels of POPs to food sources. The study area comprises three zones that coincide with these two high-density nuclei. Pellets and feathers were collected. Pellets were used to determine the composition of the diet. Feathers were used to determine levels of stable isotopes and POPs. We analyzed 18 PCB congeners, 6 DDTs isomers, 27 PBDE congeners and 2 DP isomers. Diet analysis, showed that cattle was the main source of food and significant differences between the isotopic signals of C and N in central Chile and the southern areas, where the central population presented enriched values of C and less than N. Regarding the compounds analyzed, only 32 were found above the limit of quantification. The families of contaminants best represented were PCBS, DDTS and PBDES, being the 91% of the compounds found. Our results pointed that C signal provides a good proxy of latitudinal feeding zones/ranges in Andean Condor, an influential factor in terms of pollutant emissions; while the differences in the N signals evidence dietary differences in terms of trophic position.

MO298

Food web dynamics of per- and polyfluoroalkyl substances and mercury in the Greenland Sea, Northeast Greenland

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The Arctic has turned into a sink for various anthropogenic compounds such as persistent organic pollutants (POPs) and mercury (Hg) that tend to biomagnify and consequently cause toxicity in a wide range of higher trophic species. Nonetheless, new chemicals with POP-like chemical properties remain unregulated and continuously enter into the environment. Among these, several per- and polyfluoroalkyl substances (PFASs) are of special interest as they have been found widely in Arctic species. Among the PFASs, only the production and consumption of perfluorooctane sulfonate (PFOS) is currently restricted under the Stockholm Convention for POPs. Similarly, Hg, also being widely documented in Arctic biota and considered of major toxicological concern, has recently been restricted globally, under the Minamata Convention. The objective of the present study is to provide the first assessment of food web biomagnification of PFASs and Hg in the Greenland Sea off Northeast Greenland, a pristine region currently facing rapid environmental change. The food web investigated spans from primary consumers, i.e. *Calanus* spp., over benthic and pelagic invertebrates and fish, up to seabirds. The relative trophic position was determined using bulk tissue stable nitrogen isotopes (δ^{15}N), and we will present several biomagnification proxies to quantify and characterize the food web dynamics of the targeted compounds. Preliminary results show increasing Hg concentrations along the food web from *Calanus* spp. (median=0.03 μg g^{-1} dw; range=0.03–0.07 μg g^{-1}dw), being assigned trophic level 2.0, over other invertebrates (median=0.04 μg g^{-1} dw; range=0.01–0.25 μg g^{-1}dw), feeding at trophic levels ranging from 1.8–2.8, over several benthic and pelagic fish (median=0.12 μg g^{-1} dw; range=0.10–0.15 μg g^{-1}dw), feeding at trophic levels ranging from 2.7–3.3, up to the highest concentrations in the seabird species (median=0.31 μg g^{-1} dw; range=0.11–0.50 μg g^{-1} dw), feeding at trophic levels ranging from 3.3–4.0. We will present a more detailed assessment of Hg and PFAS dynamics in the Greenland Sea food web, thus providing the first comprehensive and quantitative baseline study for this pristine though rapidly changing Greenlandic food web.

MO299

From population level effects to community functioning: a network approach

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Assessment of the ecological recovery of populations after exposure to environmental stressors (e.g. plant protection products) is, so far, abundance-based only: if after a certain interval, untreated and treated study sites don't show differences with respect to the inhabiting populations, recovery is assumed. Studying abundances of species populations in isolation does not enable the behaviour of the ecosystem to be predicted, as often emergent or counterintuitive effects can arise from the myriad of pathways of interactions. A shift in focus from species to interaction networks is necessary to achieve sustainable pest management, to conserve ecosystem processes and ultimately landscape-scale delivery of ecosystem services. Network approaches provide a powerful presentation of the pattern of interactions among species, highlight their interdependence and help to make inferences about effects of observed disturbances on species composition, functionality, resilience and ecological recovery of communities. We populated a network model with abundance data of a natural non-target arthropod community obtained from a hay meadow. Using a machine learning approach, we constructed a co-occurrence network. After validation, we evaluated network metrics, like the percentage of taxa that were trophically top (taxa with "no" consumers = top-predators), intermediate (taxa that both consume and are consumed), and basal (taxa that do not consume other arthropod taxa, but are e.g. herbivores), connectance (the proportion of possible links between species that are realized) and modularity (subsets of species (= modules) that are internally highly connected). Furthermore, we investigated the importance of the interacting taxa for the robustness of the overall network. In a subsequent step, we disturbed the network (reduced abundance of certain taxa) and evaluated the network metrics in comparison to the results of the undisturbed network. This yielded indexed measures of functionality, resilience and ecological recovery of the manipulated network. Several important network features, like connectance, modularity and robustness changed due to the disturbance. With this method, we achieved a ranking or categorization of effects on single populations with regard to their impact on the overall ecosystem robustness and functioning. We conclude that network approaches are an important tool to interpret the results of higher tier field studies for environmental risk assessments.

MO300

Mercury accumulation in the trophic webs in a tropical estuary: Cartagena Bay, Colombian Caribbean

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The metil-mercury bioaccumulation process is determined by throphic position and the feeding habits of species. At Cartagena Bay there are trophic webs based on detritus and phytoplankton that may show different mercury bioaccumulation patterns, which were tested at different climatic seasons and bay areas at the present study. Five field trips at different rain levels and at five bay areas each time were carried out in order to collect fishes of different trophic levels, phytoplankton samples and detritus. A positive correlation between the total mercury concentration (HgT) and the metil-mercury (MeHg) concentration in the phytoplankton was found ($r=0,77$; $p< 0,0001$). All the fish species collected accumulated total mercury, from which, 10 species had levels of this metal higher than those permitted by the WHO for vulnerable communities. Not only the carnivorous had higher levels of mercury than planktivorous and detritivorous fishes but also the bigger the fish the higher the total mercury level as expected. The metil- mercury level in phytoplankton was 22%, whereas in zooplankton was 23% and in fishes more than 50% of the total mercury. The metil mercury is accumulated more efficiently by the planktivorous pathway than the detritivorous pathway.

MO301

Riparian Spiders in Ecotoxicological Studies

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A major first level inquiry for many environmental contaminant studies is to determine whether exposure is occurring in the aquatic or terrestrial ecosystem. More complex scenarios, however, can occur when contaminants and organisms are exchanged between linked aquatic-terrestrial systems. Historically, ecotoxicological studies operated under the assumption that once a contaminant enters the aquatic ecosystem, it is primarily an aquatic concern only and that export to the terrestrial ecosystem is insignificant (i.e., aquatic systems are sinks for contaminants). Early interchange studies highlighted the importance of contaminant transport in the form of cross-system biotic subsidies, for example emerging aquatic insects or salmon carcasses. More recently, riparian spiders have gained traction as a valuable tool in this field of study. These abundant and widespread predators feed on aquatic emergent insects and can act as a bridge to the terrestrial food-chain. Their life history characteristics (e.g., ubiquitous distribution, small home ranges, short life spans) make them attractive as potential model organisms. Progressively, more studies are using riparian spiders to investigate a variety of topics related to contaminant fate and exposure, including food-chain dynamics and contaminant monitoring and export. Here we overview key studies utilizing riparian spiders as environmental indicators, current research foci, and future research directions.

MO302

SYSTEMLINK - a new project on the effects of stressors across ecosystem barriers.

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The impact of matter input from terrestrial sources on aquatic systems is well known. The reverse process, i.e. the transport from water (source) to land (sink) in aquatic-terrestrial meta-ecosystems, has received less attention. In SYSTEMLINK, we focuson the bottom-up and top-down mediated interactions in terrestrial ecosystems, which propagate from aquatic environments as a result of their exposure to anthropogenic stress. We consider micropollutants (fungicides and insecticides) and invasive species (riparian plants and invertebrates) as important manifestations of multiple stressors in disturbed aquatic ecosystems. We hypothesise that 1) invasive invertebrates and insecticide exposure and 2) invasive riparian plants and fungicide exposure cause top-down and bottom-up mediated responses in terrestrial ecosystems, respectively. We test these general and several more specific hypotheses through collaborative experiments in replicated outdoor aquatic-terrestrial mesocosms (*site-scale*) amended by joint pot experiments (*batch-scale*), field studies (*landscape-scale*), and modelling. All experimental setups will be derived from the landscape scale representing a multi-stress environment. Several scales will regularly be combined to overcome scale-specific restrictions and to ensure both cause-effect quantification as well as environmental relevance of the results. Ultimately, SYSTEMLINK thrives to increase our knowledge on effect translation across ecosystem boundaries. By integrating biogeochemical fluxes and biological subsidies we will be able to quantify their relative importance. Furthermore, we will closely combine the often-separated aquatic and terrestrial research areas.

MO303
The pollution driven bacteria and ciliate community structure in a highly impacted microbial loop-based freshwater river ecosystem
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The Wen-Rui Tang River is an important river system in Wenzhou, Zhejiang Province, China and is considered the mother river of the city. Historical use and modern industrial activities have resulted in the river becoming severely polluted. It has become one of Wenzhou's largest pollution sources and one of the main pollution distribution centers, directly affecting the day-to-day life of Wenzhou citizens. To understand the ecological impacts of the pollutants, this projected assessed the water quality and ecosystem structure of the Wen-Rui Tang River as a function of time and land use over an 18 km reach of the river as it transits Wenzhou from the agricultural areas at the boundaries of the city, through the Sanyang Wetland and the urban core. Based upon current and previous work, the primary pollutants for the river have been shown to be organics such as total phosphorus and total suspended solids. These pollutants have had serious deleterious effects on the aquatic ecosystem. The reduced light penetration due to excessive suspended solids and the high organic loading has created a microbial loop trophic system. This project assessed the temporal and spatial distribution of bacteria and ciliate populations in the Wen-Rui Tang River system in order to develop an understanding of the microbial community of the ecosystem. The data detail the distribution of microbes as a function of water quality and provide a basis for understanding the structure of the microbial loop trophic system. Correlation of the bacterial and ciliate populations with pollutant levels and land use provide a means to develop ecosystem models to be used as a tool to guide remediation of the Wen-Rui Tang. The data presented here forms the basis to develop a model of a freshwater microbial loop ecosystem. The model developed with this data will enable more accurate targeting of remediation strategies to rehabilitate the Wen-Rui Tang, which is at the heart of Wenzhou history and culture.

MO304
Tracer or poison: is labelling with stable isotopes safe for aquatic food webs?
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Heavy stable isotopes (such as ^{15}N) are commonly used to trace the flow of energy, nutrients, and matter in food webs based on the assumption that their enrichment does not affect the physiology of consumers. However, a recent study (Andriukonis & Gorokhova, 2017, Scientific Reports) implies that growth of algae was negatively correlated with ^{15}N concentration in medium, while at an intermediate level of labelling (3.5 at%) growth lag phase was shortest (effect size [ES] ~10%). To test if this is a universal phenomenon in aquatic food webs that would rule out the use of ^{15}N as tracer, we performed two experiments. In the first one, we tested if consumption of a labelled photoautotrophic resource does affect the survival and fitness of an aquatic primary consumer. We cultured algae (*Desmodesmus subspicatus*) at differing ^{15}N concentrations (0.37-50 at%) before they were fed to *Daphnia magna* during a 21-day lasting reproduction test. The experiment followed in general OECD 211 but with 6 animals cultured in 150 mL medium with a replication of five. In the second experiment, we tested if heterotrophic microorganisms show similar effects as observed for algae. Therefore, black alder leaf material was subjected to colonization and decomposition by a natural microbial community (particularly fungi and bacteria) for 12 days with medium containing increasing ^{15}N concentrations (n=15). During the first experiment, reproduction was not affected (ES < 5%). However and contrary to literature, mortality data indicate the ^{15}N labelling at 3.5 at% to be rather unfavorable with survival being reduced by 10% compared to the control. This observation is supported by the leaf decomposition being lowest for this treatment during the second experiment (ES >10%), although differing non-significantly to the control (i.e., 0.37 at%). Currently, we are analyzing preserved samples of both experiments (e.g., physiological condition of daphnids and microorganisms) to verify the identified trends (i.e., detrimental implications in the survival of a primary consumer and the functioning of microbial decomposers at 3.5 at%). As the present and literature data indicate, nonetheless, that relevant heavy stable isotope concentrations (i.e., up to 35 at%) do not affect biological and ecological responses considerably, labelling with ^{15}N still seems well-suited to trace the flow of energy, nutrients, and matter in aquatic food webs.

MO305
Tracing indirect effects: stable isotope and elemental analysis as state-of-the-art tools
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Changes in trophic interactions or food web structure are hard to detect using traditional approaches. Stable isotope signatures and elemental composition, which both provide information on the assimilated resource, appear as promising tools. To test for their suitability in an ecotoxicological context, in a first experiment, the omnivorous amphipod *Gammarus fossarum* was given the choice to feed either on black alder leaves or prey on mayfly larvae (*Baetis* sp.). This trophic interaction was assessed under exposure towards the neonicotinoid thiacloprid (0.75 μg/L). The study followed a multi-factorial design (thiacloprid x *Baetis* x leaves) to assess the effect of all factors – individually and in combination. Consumption of *Baetis* and leaves were monitored for two weeks and stable isotope signatures and elemental composition were determined for both resources as well as the predator (i.e., *G. fossarum*) at the termination of the experiment. In a second experiment, we tested if chemical stress could affect stable isotope signatures by exposing gammarids towards thiacloprid (0, 0.75, or 6 μg/L) for up to six weeks and providing them with a single food source (i.e., leaves or *Baetis*). In the first experiment, thiacloprid exposure affected the food choice of *G. fossarum*: a higher consumption of *Baetis* was observed, while this was not reflected by gammarids' elemental composition. However, the N-content of *Gammarus* was increased by thiacloprid when only fed *Baetis*. While providing valuable physiological information, elemental analysis thus seems unsuitable to trace changes in dietary interactions due to chemical stress. In contrast, mixing models based on stable isotope signatures well reflected the increased predation success and no differences in signatures were observed between exposed and unexposed animals when only leaves or only *Baetis* were offered. Moreover, the second experiment revealed that even after six weeks of exposure towards 6 μg/L, a concentration significantly reducing physiological fitness of gammarids and increasing their mortality rates, final stable isotope signatures differed only minimally (< 0.5 ‰) when compared to the control. Although further experiments with different test systems (i.e., combinations of consumers and resources as well as chemical stressors) seem necessary to draw reliable conclusions, our data suggest that stable isotope analysis is indeed a promising tool to trace indirect effects of chemical stressors in exposed food webs.

MO306
Transfer of mercury within a sediment-nettle-insect food web at a chlor-alkali landfill
L. Yung, C. Bertheau, Université de Bourgogne FrancheComté; N. Regier, University of Geneva / Department Forel Earth and Environmental Sciences; V. Slaveykova, University of Geneva / Département F.-A. Forel des sciences de lenvironnement et de leau; D. Cazaux, INOVYN France; M. Chalot, Université de Bourgogne Franche-Comté
Mercury (Hg) commonly enters ecosystems through anthropogenic sources such as atmospheric emissions or wastewater from industries. Sediment landfills from chlor-alkali industries require Hg stabilization to prevent air pollution by evaporated Hg. We recently demonstrated the relevance of phytomanagement process based on poplar plantations to revegetate and confine the Hg released from the soil in an experimental plantation located at a sediment landfill. Five years after the plantation, biotic and abiotic conditions have promoted the appearance of a spontaneous herbaceous cover highly dominated by the stinging nettle (*Urtica dioïca L.*). In natural conditions, this plant species hosts an important diversity of insects playing a key role in trophic interactions within the ecosystem. As demonstrated for aquatic ecosystems, Hg is readily biomagnified through food webs, from plants to insects, birds and mammals. The present study aimed at evaluating the trophic interactions at the nettle scale in a phytomanagement field trial and their implications for the Hg flux through a sediment-nettle-insect food web system. Insects were collected every month using a sweep net, from April to September 2017 and 2018. Once taxonomically and

ecologically characterized, the total Hg (THg) concentrations of the 18 most abundant taxa were analysed using an AMA-254 analyser. Several trophic links were identified with a ratio of herbivorous /predatory of 23 %. The mean concentrations of THg for insects collected from the experimental plantation were significantly higher than those from the control site. Within the nettle-related food web, the THg concentration followed the order of nettles (21.2 ± 1.0 μg/kg) < herbivorous (27.5 ± 4.3) < predator specialists (43.0 ± 14.1) < predator generalists (617.3 ± 47.2) showing a significant bioaccumulation. Moreover, we observed that insects that spent part of their life cycle directly on Hg sources had a higher THg concentration average (918.4 ± 80.5 μg/kg) than generalist predators of the nettle food web. Hence, our study showed a complex network of trophic interactions from the studied nettle-insect food web. Overall, taxa that contributed the most to the Hg transfer to the highest trophic links were generalist predators and those in direct contact with Hg sources. Moreover, in revegetated sediment landfills, plants did not seem to be the primary source of Hg exposure for wildlife.

MO307

Trophic transfer and insect metamorphosis decouple contaminant exposure in linked aquatic-terrestrial food webs

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Aquatic insects link food web dynamics across freshwater-terrestrial boundaries and subsidize riparian consumer populations. Contaminants can accumulate in organic matter and organisms affecting contaminant exposure and resource quality for consumers, yet the factors regulating these patterns at the land-water interface are not well understood. To test how exposure concentrations in aquatic food webs are related to those on land, we conducted a large-scale field study of the accumulation patterns of trace metals through linked stream-forest food webs in the Colorado Rocky Mountains (USA). Metals were transferred across trophic and metamorphic steps less efficiently from high metal streams, resulting in a decoupling of exposure risk between food webs in aquatic and terrestrial ecosystems. Although the relationships between consumer and resource tissue concentrations weakened with each trophic transfer, they were not lost until metamorphosis from larvae to adult. On average, metal concentrations increased with each trophic step then decreased sharply across metamorphosis from larval to adult insects. Our results indicate that insect metamorphosis and trophic processes combine to limit trace metal transfer from contaminated freshwaters. These findings have implications for managing exposure and developing riparian organisms as indicators of aquatic contamination. Furthermore, our findings bolster our working hypothesis that metamorphosis is a critical process regulating the cross-ecosystem effects of contaminants and that, being an act of self-consumption and tissue building, metamorphosis may have similar effects on insect chemistry as trophic transfer.

MO308

Trophic Transfer of Radioisotopes of the Micronutrients Manganese, Cobalt and Zinc in the Baltic Sea: A Cosm Study of a Shallow Benthic Food Web

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The Baltic Sea is regarded as one of the seas with the highest levels of radioactive pollution from anthropogenic sources. Despite this, little is known of radionuclides' ecological fate in brackish environments. In this study, a multi-species cosm trial was used to investigate the fate of three radioactive isotopes of micronutrients, Mn-54, Co-57 and Zn-65, which are regularly released into the Baltic Sea from nuclear power plants (NPPs). A parallel trial was done with C-14 to verify the carbon flow and the trophic structure of the cosms. The cosm trial consisted of exposing a benthic Baltic Sea community, consisting of the macroalgae *Fucus vesiculosus* (bladder wrack) and two grazers, the gastropod *Theodoxus fluviatilis* and the isopod *Idotea baltica*, to trace amounts of these radionuclides. With this setup several relevant factors could be studied. Primarily, adding the radionuclides to the cosms allowed for the investigation of the direct uptake from water to both algae and grazers. Secondly, the potential effects of eutrophication on the uptake of radionuclides could be investigated by exposing the cosms to different nutrient levels before the radionuclide addition. Thirdly, by having different combinations of grazers and macroalgae in the cosms before the radionuclide exposure allowed the effects of grazer-macroalgae interactions on the uptake to be studied. Finally, the difference in uptake depending on indirect exposure could be distinguished by using exposed macroalgae to feed non-exposed grazers. Overall, these factors are all relevant by themselves and the combination of them through this study provides a way to detect any synergistic effects on radionuclide uptake in the benthic community between these factors. These approaches also allow this study to identify and verify trophic links and consequently the ecological fate of the three radionuclides. Results from the cosm trials showed that uptake from water of all radionuclides occurred in *F. vesiculosus*. Furthermore, radionuclide uptake was observed in *T. fluviatilis* and the isopod *I. baltica* both when exposed to water and when feeding on exposed *F. vesiculosus*. These results will be of importance for further risk assessments of NPP-derived radionuclides and could indicate priority targets in NPP waste water treatment.

From Hazard Assessment to Regulatory Risk Management Action - Approach Development for UVCBs (P)

MO309

The Safety Assessment of Natural Complex Substances: Program Development

D.T. Salvito, Research Institute for Fragrance Materials (RIFM) / Dept of Environmental Science

Natural Complex Substances (NCSs) are plant extracts prepared by a variety of processing methods (e.g., steam distillation) and used in perfumery. In a regulatory context, these would be considered multiconstituent substances (MCSs) or unknown, variable composition, or biological substances (UVCBs). The Research Institute for Fragrance Materials has established an ongoing safety assessment program addressing human health and environmental risk of ingredients utilized in the preparation of fragrance compounds used in a variety of consumer and personal care products. The next phase in this program is the assessment of NCSs. A framework has been developed addressing the applicability of endpoint evaluation based on whole substance, blocks, or individual components. These endpoints include environmental hazard (i.e., PBT evaluation) and risk, skin sensitization, phototoxicity, genotoxicity, respiratory, repeat dose, and reproduction. Additionally a library of terpene blocks and methodology for grouping related NCSs to facilitate this assessment process has been proposed. NCSs should be able to be grouped based on the plant type, the part the NCS is extracted from (e.g., fruit, branches), how the part is processed, and the chemical composition of the extract. As these safety assessments are developed and published they will be publicly available at the Food and Chemical Toxicology Fragrance Material Assessment Center (http://fragrancematerialsafetyresource.elsevier.com/).

MO310

From mapping to identification: Paving the way to UVCB risk assessment

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Risk assessment of complex substances such as multi-constituent substances (MCs), and substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs), presents numerous challenges. International regulatory programs (specifically REACH, Canada's Chemicals Management Plan, and USEPA's PMN process) have highlighted the complexities of characterizing fate and exposure, risk assessment, and registration of these types of substances. This is why HESI's UVCB Committee is working on developing approaches for assessing ecological risks of UVCBs to meet regulatory needs. A grouping framework was created to map the vast and complex universe of UVCB substances according to chemical and/or functional classes, exposure and use patterns, production volume, potential toxicity and other criteria. This framework is the foundation of all work performed on this project. It provides a better understanding of the nature of these substances and their sector of use, as well as some insight on which substances present the most significant regulatory challenges. In a second step, the Committee will develop identification and characterization strategies for the mapped substance groups. For each group, the overall goal is to determine the minimum level of information required to perform a robust and fit-for-purpose ecological risk assessment. To do so, the Committee has been exploring various issues including definitions of acceptable levels of uncertainty and variability when assessing risk and strategies to characterize substances with limited or no structural information; and development of criteria that could be used in a weight of evidence approach to substance identification and characterization. Characterization approaches have included discussions around how knowledge of source material and production processes can help inform substance identification. This two-pronged approach is expected to ensure that the efforts and resources deployed for UVCBs risk assessment match actual needs, and help streamline the risk assessment process. Examples of UVCB mapping will be presented and some of the most common categories of UVCBs will be highlighted along with the associated assessment challenges. Substance identification and characterization methods will be illustrated, as well as how they provide the necessary information for a reliable risk assessment. [The views of the authors of this presentation are those of the authors and do not represent the views of their respective organizations]

Ecosystem Services: Progress, Case Studies and Reflections (P)

MO311
SETAC Ecosystem Services Interest Group
S.E. Apitz, SEA Environmental Decisions Ltd
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MO312
SETAC Ecological Risk Assessment Interest Group
L. Burgoon, U.S. Army Engineer Research and Development Center / Environmental Laboratory
.

Modelling and Monitoring of Pesticides Fate and Exposure in a Regulatory Context (P)

TU001
COMPARISON OF SOIL PHOTOLYSIS IN DRY AND MOIST SOIL LAYERS
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The OECD guidelines for the testing of phototransformation of chemicals on soil surfaces are a draft document from January 2002. The guideline suggests two types of soil layers may be used for investigation, one maintained at soil moisture of approximately 75% of field capacity and one allowed to dry out completely. When a test substance is applied to soil for investigation into phototransformation, the results obtained can vary significantly depending on whether it is applied to moist or dry soil layers. Though the rate and method of phototransformation is dependent on the nature and structure of the test substance, there may be trends observed between test substances for the different types of soil layers. Whilst the OECD guideline remains draft, there is opportunity to consider and potentially adapt, the methodology used in soil photolysis investigations. Whilst SETAC 1995 is still the recommended guideline for PPP's, it is increasingly common for studies to be conducted using both moist and dry soil layers. In addition to the obvious cost and timeline implications, the different designs could lead to more extensive issues e.g. different degradation rates and pathways. This poster examines the similarities and differences in phototransformation routes and rates when the same compound is applied under moist and dry soil conditions.

TU002
Field Soil Degradation under Exclusion of Surface Processes, Comparing Sand Cover against Incorporation of the Test Substance
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The degradation of a potential volatile plant protection product (vapour pressure > 1 x 10^{-4} Pa at 25°C) under field conditions was investigated at four sites in Europe. Two trials were performed in Germany, one trial was performed in France and one in the UK. Applications were conducted on bare soil in August or September. Following application of the substance, two of the following procedures were compared to eliminate the impact of surface loss processes (e.g. photolysis, volatilization) on the $DegT_{50matrix}$ value as recommended in the OECD Guidance document for Conducting Pesticide Terrestrial Field Dissipation Studies:[(1)] Even application of a layer of commercial fine sand to the soil surface, achieving a depth of at least 3 mm, although not recommended by the OECD Guidance if the substance has a vapour pressure > 1 x 10^{-4} Pa, unless other experimental evidence is available that volatilization is not a route of dissipation. Incorporation of the substance in the soil immediately after spraying to the soil surface and mixing over a target depth of 7 cm. A plot power harrow was used for the application and after harrowing, the plots were rolled. The first soil sampling was done directly after the applied procedure. No tillage or fertilization was performed in the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation. Soil specimens were taken down to a soil depth of max. 50 cm, cut into sections of 10 cm, extracted with appropriate organic solvents followed by LC-MS/MS analysis with a limit of detection (LOD) of 0.01 mg/kg for the test substance. The results show no significant differences of the employed procedures on the soil degradation/dissipation of the test substance. The application of a layer of fine sand to the soil surface is already sufficient to prevent dissipation of the applied test substance with a vapour pressure of > 1 x 10^{-4} Pa. [(1)] OECD Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, Series on Testing & Assessment No. 232, Series on Pesticides No. 82, March 04, 2016, ENV/JM/MONO(2016)6

TU003
Benefits of the new EFSA guidance on soil exposure compared to current regulatory procedure
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1. Introduction Following MS consultations EFSA initiated a revision of the guidance for the soil exposure assessment and soil persistence. The EFSA soil exposure guidance (GD) covers applications of plant protection products to annual- and permanent crops [1]. The exposure assessment targets obtain a 90^{th} percentile of the PEC in space and time in the selected regulatory zones North, Centre and South. The calculated exposure concentrations will be used for risk assessment of soil organisms living in the soil. 2. Comparison of EFSA GD with current regulatory procedure The EFSA guidance introduces a tiered approach with increasing realism from lower to higher tiers for exposure refinement when needed. Pre-defined scenarios have been provided for the first tier for each regulatory zone; these can be used for both EU- and zonal assessments. For Member State (MS) assessments, an EU MS can be selected as well. The new approach also takes into account realistic description of foliar wash-off from the crop canopy. Further the soil density, average annual temperature and rainfall are taken into account allowing natural variability to be considered. The new approach introduces options for considering the type of application, e.g. spray applications or incorporation into the soil. Non-homogeneous treatments such as in-row and between-row applications can be included as well. As a higher tier, the guidance allows users to generate own data on plant protection products for improving the canopy wash-off processes. The EFSA GD is supported by user-friendly software tools where PERSAM provides quick solutions to Tier-1 and Tier-2 while the numerical models PELMO and PEARL can be used for refinement of the exposure assessment at Tier-3. The models also provide options for the user to perform the complete soil risk assessment and can produce complete output reports for regulatory submissions. 3. Conclusions The EFSA GD on soil exposure and the supporting software tools take EU wide environmental variability into account and provide a substantial scientific improvement compared to the current regulatory procedure. The new guidance is expected to be implemented for regulatory use during 2019. 4. References [1] EFSA. 2017. EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2017;15(10):4982. DOI: 10.2903/j.efsa.2017.4982

TU004
Exposure Assessment of Residual Organochlorine Pesticides (OCPs) in Upland and Pasture land Soils in Korea
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Residual organochlorine pesticides (OCPs) are chemical substances that are resistant to environmental degradation chemical, biological and photolytic process, and are bioaccumulated with potential significant impacts on human health and the environment. The majority of OCPs were designated as persistent organic pollutants (POPs) by the international community at the Stockholm Convention on Persistent Organic Pollutant. This study was conducted to investigate the OCPs residue in paddy and upland soils. Extraction and clean-up method for the quantitative analysis of OCPs was developed and validated by gas chromatography (GC). Recovery and limit of detection (LOD) of OCPs in soil were 74.4-115.6 and 0.02-0.08 g/kg, respectively. The precision was reliable since RSD percentage (0.5-3.5) was below 20, which was the normal percent value. The residue of OCPs in pasture land and upland soils was analyzed by the developed method, and α-endosulfan, -endosulfan, and endosulfan sulfate were detected at 0.36-87.16, 0.06-179.46, and 2.44-287.16 g/kg, respectively. The detection frequency of three compounds were 7.8%, 16.9% and 29.9 among 109 samples, respectively. But OCPs in onion, carrot and radish, which are cultivated crops in OCPs detection soil, were not detected. These results showed that the residue in paddy and upland soils were lower level than bioaccumulation occurring.

TU005
Comparison of the measured and predicted pesticide concentrations in soils of Russia
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Mathematical modeling is an efficient technique to predict pesticides behavior in soil, air, water and groundwater. The main advantages of mathematical modeling include efficiency, time saving and ability to cover a large variety of pesticides application in natural conditions. Mathematical models as an instrument to assess the pesticides concentrations in environment are widely used in European Union. Currently, the pesticides behavior predicting based on the PEARL model designed in the EU as well as the standard Russian scenarios are a part of a multilevel risk assessment of the pesticides, being registered in Russia. The simulated pesticides concentrations are used for the environmental risk assessment of pesticides to non-target organisms. The environmental risk assessment is calculated as the ratio between the acute toxicity or chronic toxicity LC_{50}(NOEC) and pesticide concentrations in soil, surface and ground water. This paper attempts to answer the question: may the predicted concentration be compared with the measured concentration and be applied for environmental risk assessment? The aims of this study were to 1) *determine* the pesticides residues in 3 types of soils under field

conditions; 2) compare measured and simulated pesticides concentrations in soils; 3) evaluate the *quality of mathematical modelling to predict pesticides concentrations; 4) illustrate the application PEC in soil for environmental risk assessment of 6 pesticides to the earthworms.* The concentrations of lenacil, metsulfuron-methyl, triallate, pyrimethanil, fenpropimorph and chlorantraniliprole were determined during the period from 1999 to 2011 in the soils of Moscow, Kursk and Saratov regions. The predicted concentrations of these pesticides were calculated by model PEARL with the standard scenarios for the mentioned regions. The comparison of the measured and predicted data showed that the model results generally agree with the pesticide concentrations under field conditions. The model PEARL may be recommended as the efficient tool to predict pesticides behavior in soil. The risk evaluation of pesticides to earthworms are commonly based on the ratio of the acute toxicity or chronic toxicity LC_{50}(NOEC) and the pesticide concentrations in soil. The acute effects of the studied pesticides to earthworms were negligible. But the application of pyrimethanil and fenpropimorph could have the negative implications for the growth and reproduction of earthworms.

TU006
Identification of groundwater monitoring web for the investigation of the presence of residues of 1,3-dichloropropene and related compounds in Italy
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In Italy, the use fumigants in on bare soil, pre-planting/sowing of the crop, is widely consolidated in agricultural practices, particularly in horticulture. 1,3-dichloropropene (1,3-D) has been used to control nematodes in agriculture since the 1960's, it is therefore a very well-known compound and a number of studies describing its environmental fate and effects are available in literature. The prevention of groundwater contamination from 1,3-D has often been a subject of interest including from a regulatory point of view. In a previous work, areas vulnerable to the leaching of 1,3-D were identified in Italy. The percent of sand content was selected as the key parameter to classify the soils, the area being considered as vulnerable where sand content in soil was >80%. This choice was based on the label recommendations for the commercial products containing 1,3-D, i.e. risk mitigation measure *Do not apply in soil with a percentage of sand >80%*. In this work, we proceeded with the identification of a suitable set of groundwater wells to be included in a monitoring program, starting from the areas previously identified. The attention was focused on two provinces, one in Sicily (Ragusa) and one in Veneto (Verona), located in South and North Italy respectively, and representing different crops and agronomic practices (e.g. greenhouse and open field). Suitable wells were identified using information about geolocalization, structure and availability, and an onsite survey to check the local conditions at wells location. The final identification of the groundwater wells was based on the following criteria: location of wells in areas where 1,3-D is utilized, hydrogeological conditions; accessibility to selected areas; operating conditions of wells; and depth of water. During the sampling phase, wells were characterized by determining their precise location on a map, recording generic information on wells, as well as measuring a set of parameters such as pH, redox potential, conductivity, dissolved oxygen, and turbidity. Water samples were analyzed for the presence of 1,3-D and process impurities. Preliminary results show no to very low residue of the measured compounds. Final results will be presented and discussed. All the critical issues encountered during the wells identification and sampling phases will also be discussed.

TU007
Developing a MACRO meta-model for Swedish drinking water abstraction zones
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In Sweden farmers are legally obliged to apply to local authorities for permits for pesticide use if their land lies within a designated water abstraction zone. To facilitate risk assessment and decision-making for water abstraction zones, a web-based decision support tool is being developed by SLU. The tool, which is to be used both by local authorities (who make the decisions) and farmers/landowners and consultants, will comprise a meta-model of the well-established leaching model MACRO. In a first step, ca. 19000 leaching simulations were performed with MACRO 5.2 for a pilot region in Sweden (SW Skåne). The simulations constitute 39 soil scenarios (defined by substrate type, soil texture and organic matter content), 1 climate, 1 crop, 3 application seasons and 160 dummy compounds (cominbations of Koc, DegT50 and Freundlich exponent). The aggregated output variables to be used for meta-model development were total leaching flux and total percolation over 20 years at 2 m depth, as well as overall mean flux concentrations. In a second step, a tool for meta-model development using CART (Classification and Regression Trees) was implemented in R. Subsequently, the meta-model was calibrated and evaluated using a cross-validation analysis. The poster will discuss the results of the calibration and validation exercise. Future plans include the extension of the analysis to other climatic regions in Sweden as well as the development of a spatially distributed version of the decision support tool to support water authorities working with the EU Water Framework Directive.

TU008
GeoPEARL UK - a fully spatially-distributed, higher-tier modelling approach for refining UK groundwater exposure estimates
J. Carnall, G. Hughes, Cambridge Environmental Assessments
Limited options are currently available within the UK regulatory process for higher-tier refinement of groundwater exposure estimates for plant protection products (PPPs). Hughes (2015) previously reported a higher-tier modelling approach for assessing the exposure of groundwater in the UK to PPPs at Tier 3b of the FOCUS assessment scheme (FOCUS, 2009). This approach incorporated elements of both the UK higher tier drainflow and NL GeoPEARL assessment frameworks, pairing 15 UK soil classes with 4 representative weather stations (dry, moderate, wet and very wet climates) in a series of 30-year simulations using the FOCUS-PEARL and FOCUS-MACRO models. The results of these simulations were weighted according to the extent of crop production on each soil/climate combination, and a cumulative frequency distribution was produced, from which the 80th spatial percentile PECgw value was derived. In this poster we present a fully spatially-distributed, higher-tier modelling approach for the UK, to assess the risk posed by PPPs to groundwater at Tier 3b of the FOCUS assessment scheme. Climate files suitable for use with the FOCUS-PEARL model have been derived from MARS weather data (provided by the Joint Research Centre at 25 km resolution), and typical crop rotations for each soil type and region have also been included. The cumulative frequency distribution resulting from the spatially-distributed modelling approach is compared with corresponding results from the standard FOCUS groundwater scenarios relevant to the UK, and the refinement provided by the spatially-distributed approach is assessed. **References:** Hughes (2015). Development of a UK higher tier groundwater environmental risk assessment for pesticides. Poster presentation at SETAC conference, 2015. FOCUS (2009). Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU. Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 1, 604 pp.\n

TU009
Using modelling approaches to transfer results of groundwater monitoring studies to other regions of interest
N. Kehrein, knoell Germany; S. Mayer, Bayer AG / Crop Science Division; R. Sur, Bayer AG - Crop Science Division / Environmental Safety
Groundwater monitoring is part of the European risk assessment scheme for the (re-)authorisation of plant protection products (PPP). Its main objective is to preclude negative effects on humans, animals, and the environment by confirming compliance of active substances and their metabolites with appropriate protection goals in groundwater. Conducting groundwater monitoring is complex, labour intensive, and usually requires several years of sample collection. The number of monitoring sites within a study is limited by the associated costs and required effort to ensure high study quality. It is therefore often necessary to focus monitoring on the main regions of product use. Spatial modelling approaches can be employed to set results of monitoring studies into context and to compare them with other regions which are not covered by monitoring. By using in-silico methods, the vulnerability of occurring environmental conditions to PPP leaching to groundwater is estimated and compared. Vulnerabilities are based on spatially explicit exposure estimates calculated by process-based models such as FOCUS PEARL. Monitoring results can then be transferred and mapped to other regions if it can be shown that existing monitoring sites are representative for their respective environmental conditions. The presented approach is cost-efficient and creates a mechanistic link between pedoclimatic conditions and groundwater vulnerability. It reduces uncertainty by considering the natural variability of environmental parameters. It provides the means for the transfer of monitoring results from one region or country to another without the need to conduct new groundwater surveillance. The estimated groundwater vulnerability can also be used to support the selection of suitable monitoring locations.

TU010
Modelling options to assess hydraulic connectivity of agricultural fields with monitoring wells for edge-of-field groundwater surveillance
N. Kehrein, W. He, knoell Germany; F. Hegler, DR. KNOELL CONSULT GmbH; R. Sur, Bayer AG - Crop Science Division / Environmental Safety
Groundwater monitoring for residues of active substances and their metabolites is an important part of the European risk assessment scheme for the (re-)authorisation of plant protection products (PPP). In case of edge-of-field studies, monitoring wells for sample collection can be placed at various distances to test fields treated with PPP depending on the purpose and corresponding design of a groundwater monitoring study. A common challenge for the design of monitoring

studies and the interpretation of monitoring data is the need to demonstrate that well and filter screen are appropriately placed in order to detect potential residues. This is mainly determined by the hydraulic connectivity which depends on the well's location, filter placement in the saturated zone, and other environmental factors and properties. We evaluated two options to assess the hydraulic connectivity with the help of computer models. The first option uses Darcy's law and piston flow dynamics to estimate a catchment that has a hydraulic connection to the filter screen. It generally requires only few parameters which might be available as part of the monitoring study or which can be estimated with sufficient accuracy. The second option employs a two dimensional representation of the aquifer using the dynamic OpenGeoSys model. Its scenarios can be set up with a varying degree of complexity in order to accommodate different levels of data availability. The influx of leachate and PPP residues crossing the aquifer's upper boundary are simulated with FOCUS PEARL, a one dimensional process-based model for the unsaturated zone. Two dimensional aquifer simulations provide additional insight into dispersion behaviour and influence of time-dependent groundwater recharge on substance transport. The described modelling options could be used in a tiered or combined approach to assess hydraulic connectivity and support the interpretation of groundwater monitoring studies.

TU011

Work of a SETAC Group to Develop the Scientific Basis for Guidance for Regulatory Groundwater Monitoring of Crop Protection Products and their Metabolites in Europe

A. Boivin, ANSES / U3EIV; A. Gimsing, Ministry of Environment and Food of Denmark

Groundwater monitoring is considered a higher tier assessment in the regulatory groundwater assessment of crop protection products in Europe, but little guidance has been provided to date on study designs. The SETAC EMAG-Pest GW group (a mixture of regulatory, academic, and industry scientists) in 2015 began developing the scientific basis for guidance for use by regulators and industry scientists. The work of SETAC EMAG-Pest GW is now being considered for publication and should be published ASAP. In this work, it was considered that rigid study designs are not appropriate since the study design needs to be tailored to the specific protection goal being addressed as well as study objectives, which may also vary. Study designs should consider environmental conditions, the properties affecting environmental behaviour of the substance being studied, and site and use conditions. To illustrate how study design can vary, the group has proposed general study designs for seven hypothetical exposure assessment options, ranging from protecting all zones of saturation below the soil surface to only groundwater used to supply drinking water. Designs include recommendations on in-field and edge of field studies, as well as studies focused on catchments and aquifers. Examples of potential designs and recommendations on the use of publically available monitoring data has also been included. Also general recommendations on well installation and sampling procedures have been provided. Methodology has also been developed for assessing the relative vulnerability of agricultural regions and the vulnerability of specific sites for use in study design and site selection. This work aims to facilitate the assessment of groundwater monitoring as higher tier and/or its design, mainly by presenting different exposure assessment options.

TU012

Development of a Harmonized Protocol for Measurement of Foliar Wash-off Coefficients: First Results

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The foliar wash-off coefficient is a parameter in FOCUS ground and surface water modelling which can refine the soil loading after a sprayed application of pesticides. EFSA accepts that the modelling default can be superseded by experimental data with plants under a range of relevant conditions. Previous attempts to generate such data have suffered from the lack of a harmonised protocol for the study conduct. Specifically, the selection of crop types and growth stages was not deemed suitable to cover the submitted GAP. Furthermore, the analytical methods were not always validated appropriately. An ECPA workshop in 2015 proposed that the most suitable first step in development of a harmonised protocol would be to agree the design of a "single test" (i.e. a single crop/growth stage/AI/formulation) and conduct a ring test on this design. The purpose of the ring-test would be to establish the robustness of the design in several laboratories and, importantly, to understand the impact of the specific rainfall delivery system used; i.e., whether a highly sophisticated system is necessary or whether a simpler system could be adequate. The concept of the proposed design is that whole plants are used (rather than individual leaves) to retain as much realism as possible. The wash-off coefficient is then determined by comparison of compound residues in two sets of plants (with and without a defined rainfall event) measured using a GLP validated crop residue method. Appropriate control sets will be employed to determine the extent of other dissipation processes. This initial ring test is under way using tebuconazole (Folicur EW 250) sprayed at 100 g ai/ha onto tomato plants at BBCH25. Each participant will measure the residues before and after a rainfall event of 20 mm/hour for 1 hour and will calculate the wash-off coefficient from these data. Rainfall droplet size distribution and intensity will also be measured using a Laser Precipitation Monitor (LPM) to aid understanding in the case that significant differences are observed. This poster will summarise the results obtained from the initial ring test. Keywords: *foliar, wash-off coefficient, ring test \n \n \n \n*

TU013

Understanding plant root uptake of pesticides from hydroponic solution

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Plant uptake has been suggested to influence the mobility of Plant Protection Products (PPPs). High uptake can aid with the retention of PPPs within the profile and reduce their potential to leach into potable water supplies. The suggestion of a relationship between the physio-chemical properties of a compound and the ability of a plant to take up a compound was first reported as a Gaussian-shape curve of Transpiration Stream Concentration Factor (TSCF) against the octanol-water partition coefficient (log K_{ow}). Plant Uptake Factor (PUF) was suggested as an alternative to TSCF and was based on the description of plant uptake by environmental fate models. PUF is derived from changes in the solution and therefore considers the change in concentration surrounding the plant roots from uptake over time, whereas TSCF only considers the translocation into the shoots and omits the storage within the roots. Another assumption commonly made within laboratory experiments is that crop uptake of plant protection products is passive i.e. compounds are taken up as solutes during transpiration. It also means that as the concentration increases within the plant roots and decreases in solution, the diffusion of the compound across the cell membranes should become slower. To the author's knowledge, this has not yet been tested and all previous studies have only reported a PUF/TSCF value after one time period. This experiment was set up to test whether TSCF and PUF value remain constant over a longer period, with destructive harvesting at each sampling point. This will also test the current assumption of environmental fate models where only one PUF value is required per compound. Wheat was grown in hydroponic solution and exposed to [^{14}C] 1,2,4-Triazole. They were destructively harvested at time-points of 1,2,6,12,16 and 21 days. Results from this experiment will show whether PUF and TSCF remain stable, as expected, or whether the plant uptake changes over time. This will help to determine whether the uptake of pesticides is constant with respect to transpiration for extended periods in the life cycle of a plant and therefore using a single PUF value across the crop growth cycle is correct. *Acknowledgements* – BBSRC for funding this project and Syngenta for supplying the radiolabelled compound, for the use of their laboratory and facilities and for being a CASE partner to this project.

TU014

Computational Fluid Dynamics modelling for plant canopy interception of pesticide spray droplets

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Accurately quantifying the magnitude and implications of off-target transport of plant protection products (PPPs) via spray drift is critical to evaluating ecological and bystander exposure and inferring potential spray drift impact to adjacent crops. Past work with this aim has included field, wind tunnel, and laboratory studies examining the contribution of environmental conditions, spray equipment, and spray mix (active ingredient and/or formulation) on the potential extent of off-target transport. However, there is limited research focused on better understanding factors affecting deposition on a more finite spatial scale such as the physical properties of intercepting objects that may affect directly adjacent airflow and the trajectory of the spray particles entrained. Exposure assessments for regulatory purposes typically assume 100% of drift in the vicinity of plants are captured by the canopy and do not account for the unique physical properties of individual plant species/types. Improved modelling of plant canopy interaction with PPP particles entrained in airflow has the potential to improve understanding of the relative capture efficiency of off-target plant species and to inform the PPP authorisation process. The aim of this work is to improve the understanding of pesticide spray drift particle interactions with plant canopies and the relative capture efficiency of different canopies. This presentation demonstrates an application of ANSYS FLUENT (a computational fluid dynamics (CFD) modelling tool) to estimate the capture efficiency of various plants and plant clusters receiving pesticide spray drift from applications made in a wind tunnel. Model-predicted canopy capture from two different CFD approaches are compared to measured canopy capture. The first approach includes realistic, high-resolution, 3D plant geometry generated using photogrammetry freeware. The second approach uses simplified plant geometry to greatly reduce the number of grid cells in the model domain. This approximation makes the number and extent of plants included in simulations scalable to plant clusters and potentially up to field scale. The trade-off between accuracy and complexity is also assessed.

TU015

Impact of aged sorption on drainflow and run-off losses from treated fields using the FOCUS surface water Step 3 scenarios

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The EFSA (2018) opinion on the CRD aged sorption guidance paves the way to include a relevant process for transport of plant protection products (PPP) in soil into the EU regulatory framework. Thus it can be considered a milestone to better informed regulatory decisions on exposure assessment of PPP. Both, EFSA opinion and CRD guidance are focussed on the relevance of aged sorption for leaching to groundwater where travel and residence time in soil are typically in the order of years which allows the aged sorption processes to fully evolve. In the EFSA (2018) opinion the statement is found that for entries of PPP into surface waters travel times are short so that aged soption is not relevant and should therefore not be used for PECsw. While it is reasonable to assume that the travel time to surface water is in general shorter than to groundwater, it is questionable to exclude the aged sorption process *a priori* from surface water exposure assessment. Firstly, because the aged sorption model employed accounts automatically for the effect of time (for short times the aged sorption effect will be correspondingly low). So there is no necessity to exclude its use. Secondly, residence time in soils especially for drainflow entries is typically in the order of weeks and months up to years, a time scale where aged sorption is expected to be relevant. In order to explore potential effects of aged sorption on surface water exposure FOCUS Step 3 calculations were performed with substance parameters of a test substance (ECPA07) which was used for a detailled case study in EFSA (2018). The results indicate that PECsw for drainflow entries are substantially lower when including aged sorption. Because aged sorption is generally relevant for transport processes in soils, these findings suggest the necessity to revise the proposed exclusion of aged sorption for PECsw assessments. EFSA (2018). Scientific Opinion about the Guidance of the Chemical Regulation Directorate (UK) on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. EFSA Journal 2018;16(8):5382, 86 pp. https://doi.org/10.2903/

TU016

Off-field deposition of pesticides via run-off/erosion entries coming from treated fields using FOCUS surface water Step 3

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The EFSA (2017) soil risk assessment opinion requires to include run-off/erosion entries from treated fields into off-field areas. There, it is discussed to use the 90th percentile retention efficacy of vegetated filter strips (VFS) (from FOCUS, 2007) as used in FOCUS surface water to derive the deposition of run-off/erosion entries in off-field areas. As the conservativeness of this approach is unclear EFSA (2017) recommends to re-evaluate the existing information with regard to worst case situations for off-field areas. In this work it is aimed to derive a concept for the deposition of run-off/erosion entries in off-field areas for edge-of-field situations. It is proposed to consider FOCUS surface water Step 3 for this purpose which was designed to generate realistic worst case pesticide losses from agricultural fields. As mitigation in-field VFS of low, mean and high retention efficacy are considered. The specific filter function of these VFS was derived form empirical data underlying the FOCUS (2007) retention efficacy recommendations. The mass deposited on the edge of the VFS by a run-off/erosion event was used to calculate a maximum local off-field PECsoil. Multiyear calculations allow to derive a local realistic worst case PECsoil which was here set to the 80th percentile of annual maxima. An exemplary assessment using Step 3 scenario R3, and dummy substance E showed that 80th percentile off-field PECsoil are substantially lower than the corresponding value using lower tier (Step 2) run-off/erosion losses. Conservative assessment of mitigation by VFS would consider high retention efficay for short VFS 1 m long, medium retention efficay for a VFS 4 m long and low retention for a VFS 10 m long. The approach demonstrates the feasibility to use FOCUS surface water Step 3 to define potential local off-field exposure and allows to define suitable mitigations. However, these exposure values represent a local worst-case situation which hardly affects the total population of an off-field organism under real-world conditions. Therefore, these local exposure values have to be set into context to the exposure relevant for effects at the population level as defined by specific Protection Goals. EFSA (2017). Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms FOCUS (2007). Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations

TU017

Landscape level simulation of off field exposure by runoff - Development of realistic worst-case scenarios and effectiveness of mitigation measures

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In EFSAs (2017) scientific opinion on risk assessment for in-soil organisms a preliminary approach for off-field soil exposure was proposed. Runoff is mentioned in this proposal as a major exposure route for off-field habitats. Since no suitable off-field exposure scenarios were available, generic worst-case assumptions have been proposed by EFSA (2017). Therefore, in the present study a previously initiated framework for the development of such scenarios was extended and applied to identified scenarios. In this modelling framework, the runoff from each field in a given landscape can be simulated using FOCUS PRZM. By using different deposition models, off field exposure from runoff can be simulated on the landscape level. This modelling framework combined with spatially explicit modelling, has been used to simulate off-crop exposure from runoff in generic and real-world landscapes. Additionally, the effectiveness of mitigation measures, such as in-crop buffers and in-field margins has been evaluated.

TU018

Pesticide exposure scenario for aquatic organisms in Korea - Conceptual model for protection goals

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The pesticide registration system for environmental risk assessment in Republic of Korea is currently in the process of improvement. The current scenarios for surface water are too simple to reflect reality; not take into account the spatio-temporal distribution of environmental factors in Korea and the physic-chemical properties, repeated application and various exposure routes of pesticides. This study is being phased in to improve the exposure scenarios for aquatic organisms in a more realistic way. First, one scenario for the worst case (90th percentile) was confirmed instead of scenario zones, because Korea is quite homogeneous considering agro-environmental conditions. Second, three protection goals were selected for water bodies and aquatic organisms; mudfish in paddy rice fields, aquatic ecosystem in small stream in paddy rice area and aquatic ecosystem in small stream adjacent fruit orchards. Third, ERCs (Ecotoxicological Relevant Concentration) are established for each protection goal. Lastly, the conceptual models were assumed for exposure situation of pesticides. For the next step, this study will be proceeded with data-based model development process.

TU019

Relevance of the Pesticide Application Timer (PAT) in multi-year FOCUS surface water modelling

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For the registration of plant protection products in the EU, a safe use with regard to surface water and sediment organisms? needs to be demonstrated. The FOCUS work group developed a stepwise approach. At step 3 different models for drainage, runoff and spray drift entry into different surface water bodies were combined. Due to limitations of these models, the modellin period was limited to 12 - 16 months. Currently, the possibility to run multi-year simulations at step 3 is discussed and an approach was presented by EFSA. In the present study multi-year runs were performed using AutoPEC SW to evaluate the possible consequences of an extension of the modelling period for the final risk assessment in surface water and sediment. In the draft scientific report on multi-year simulations EFSA (2018) proposed to implement a number of changes to the exposure calculation for surface water and sediment independently of the extension of the modelling period. In the present work the final concentrations in surface water and sediment (PEC) considering the same application date in each year were compared to PECs resulting from application dates selected by the Pesticide Application Timer. Therefore, final maximum PEC values as well as various temporal percentiles for the exposure assessment were evaluated using exposure profile analysis conducted with the software PEC Robot. The resulting PEC values and the consequences for the aquatic risk assessment are discussed. It turned out that weather conditions do have an impact on the amount of substances reaching surface water and sediment via drainage and runoff and hence on the final PEC_{SW} and PEC_{SED}. This is in line with previous research on application timing in PEC_{GW} modelling. Here, the difference between worst-case (rain on day of application) and realistic case (applying SW PAT rules for PEC_{GW}) was up to 20-fold. Hence, the selection of the application dates based on a Pesticide Application Timer seems to be reasonable and should be used also for the multi-year assessment.

TU020

Combining the pesticide usage in an agricultural catchment to pesticide occurrence in river waters

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The amount of pesticides used in the catchment area of river Savijoki in Southern Finland is compared to the concentration of pesticides found in stream water during years 2016-2018. The pesticide usage in the area was assessed through an inquiry targeted annually on about 80 farmers in the Savijoki area in the years 2016-2018 as a part of the SAVE project. The water samples were collected at two sampling sites from Spring to late Autumn. The results of this comparison are reported. Such results form a basis for, e.g., the evaluation of current risk reduction requirements of the used pesticides.

TU021

Towards the derivation of generic dilution factors for drinking water abstraction using landscape level assessment

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Surface water is an important source for drinking water supply in many European countries. The exposure assessment of plant protection products (PPP) at drinking water abstraction points is hence of specific interest and also a particular point in the EU regulatory framework (regulation 1107/2009). There is no generic guidance available on the derivation of a drinking water abstraction concentration. The only EU-known approach is the national approach of the Netherlands which is an integral part of their regulatory framework. This Dutch exposure assessment is simplistic but a very solid first Tier approach. It dilutes the edge-of-field PEC_{sw} considering the use intensity (most important is the cropping area within a drinking water catchment) and other dilution factors like application practice and dissipation in the water system. However, the calculation of the dilution factor so far mainly considers highly simplified worst-case assumptions. The Dutch approach assumes e.g. that all agricultural land is connected and releases water to a water body. Our work explores the dilution factors at landscape level to get a better insight into more realistic scenarios. Specifically, our goals are: (i) the derivation of a generic dilution factor on landscape level; (ii) identification of representative vulnerable drinking water catchments in the EU for generic and regulatory use. On this account we analyzed different catchments in Europe for specific crops using a GIS based approach. The focus was on catchment characteristics (crop area, soil hydraulic properties) which have a strong impact on runoff generation and therefore on the dilution of PPPs in surface water. In a first step, the potential dilution variation by crop area was investigated taking into account the stream course from headwater catchment to a larger main river. In the second step, other impacting factors (e.g. soil hydraulic conductivity) are included with the aim to explore the most vulnerable combinations of catchment characteristics for the different test catchments. The data can then be used to derive vulnerable scenarios regarding the dilution on EU level for a specific crop. It is expected that our approach leads to crop-dependent dilution factors for typical catchments in the EU that potentially can be used as a first Tier exposure assessment to derive a drinking water abstraction concentration.

TU022

A modular catchment model to support regulatory landscape-scale aquatic risk assessment

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Landscape modelling is key for future environmental risk assessment (ERA) of aquatic ecosystems related to plant protection products (PPP). Currently, local field scale models are used at lower tiers to simulate transport and fate of PPP in edge-of-field surface waters due to runoff, drainage and spray drift deposition. These local models predict the exposure concentration in predefined water bodies, i.e. streams, ditches and ponds, while neglecting the real dimension of catchments, and hence, of natural habitats of non-target species. In a higher tier approach a new generation of tools can provide more realistically relevant concentration patterns for effect and risk assessment for various biological entities, e.g. populations, communities or entire habitats. A framework for the risk assessment at landscape scale is discussed in a separate conference paper. The new approach under development operates at the catchment scale. The basis for the newly implemented tool is the hydrological programming library 'Catchment Modelling Framework (CMF)'. To allow for water and solute fluxes across entire landscapes single fields are connected to each other and to river segments by flux connections. This approach allows predicting exposure at any location in the landscape at a flexible time step. Water and solute fluxes from single fields can be either simulated by the tool or by using predefined time series. This allows the tool to use simulated outcomes from e.g. FOCUS models (e.g. MACRO, PRZM) and align with the EU risk assessment approach. The catchment tool has been connected to a TOXSWA module to simulate PPP fate for the entire catchment. A case study will be presented in which the new tool is used to simulate PPP concentrations in surface water across an entire landscape. The spatial domain is represented by the Rummen catchment in Belgium which is dominated by the cultivation of orchards. Growing pome fruit requires in that region pest management including the use of insecticides which may impact aquatic communities by spray drift input into water bodies. All minor and major river reaches are being simulated, corresponding to a total length of 150km river. Concentration patterns are provided for river reaches with a length of 50-100m, resulting from different scenarios regarding climate (wet, medium and dry) and application pattern. Uncertainties of the hydrological model affecting the assessment are identified related to structural and input data uncertainty.

TU023

Applicability of environmental emission and fate model PeCHREM/G-CIEMS for monitoring plan of paddy pesticides in water quality management

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Residual pesticide levels in river water are regularly monitored by water suppliers for water quality management of drinking water in Japan. The residual pesticides are controlled as a complementary item for water quality management with an index which is a total value of ratios of each measured concentration to each target concentration. This index should be not more than 1. Target pesticides are listed in the Waterworks Law in Japan (118 items are listed as of April 1, 2018). Water suppliers don't have an obligation, but need to determine monitoring plan which contains frequency, sampling periods, and target pesticides of the monitoring based on each area's situation, including not to monitor any pesticides. For effective and efficient management, information about temporal-spatial distributions of multiple pesticides in river water is important. We have been developing the emission estimation method (PeCHREM) for pesticides used typically in paddy fields, and the multimedia environmental fate model (G-CIEMS). The combined PeCHREM/G-CIEMS model could predict environmental concentrations of many pesticides with high temporal-spatial resolution (5km × 5km grid in atmosphere, average 9.7 km^2 of catchments, and average 5.6 km of river segments) in areas all over Japan. We have been evaluating reliability of this model from comparison of simulated concentrations with observed ones in river waters for several dozens of pesticides. This study focused on applicability of this model for water quality management, especially for prioritization of the listed pesticides for the regular monitoring. We performed constant monitoring of tap water sources at four points and river water at upper 12 points of water intake points. Finally 23 pesticides (halosulfuron methyl, simetryn, azoxystrobin, (E)-metominostrobin, fentrazamide, pyraclonil, EPN, molinate, dimethametryn, butachlor, orysastrobin, bentazon, iprobenfos (IBP), fenobucarb (BPMC), daimuron, isoprothiolane (IPT), pretilachlor, fenthion (MPP), pyroquilon, mefenacet, bromobutide, bensulfuron methyl, and MCPA) were selected as target pesticides which were detected in least one sample and were suitable for model simulation. The maximum concentrations were compared between predictions and observations for the quantified 122 combinations from total 368 combinations of 16 sampling points and 23 pesticides. The 87 combinations had a prediction error of less than one order of magnitude.

TU024

Connecting pesticide use data and environmental exposure and risk assessment

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Environmental risk assessment for plant protection products (PPPs) in Europe is performed for individual products based on the worst-case label recommended use patterns with respect to application rate, frequency and intervals between treatments, which might not necessarily reflect realistic pesticide usage. The current risk assessment frame does not take into consideration that farmers may reduce number of applications as well as application rates compared to the recommended on the label depending on the actual pest or disease pressure, but on the other hand apply simultaneously or sequentially different products during the growing season. The latter is considered a major point of uncertainty. Several guidance documents mention the need to take this aspect into account in the future and question the appropriateness of higher tier and recovery refinement options in the risk assessment of single products at present. To address concerns regarding sequential treatments, available information on realistic application practices of PPPs beyond the individual product level were analysed. The investigated data sets were collected by market research companies and are publicly (commercially) available. As a first step, we investigated generic patterns and trends with respect to the number of applications, and cumulative application rates within a season for several crops, countries, and indications. As a second step, typical spray sequences were analysed for several crop-country combinations. Typical use rates were compared to application rates on the label. The results show that actual application patterns may differ from the registered label recommendations. In many cases farmers indeed use the PPPs at rates lower than recommended and/or apply fewer treatments than maximum registered number of applications resulting in a significantly lower seasonal load of individual products and actives. These findings suggest that an additional safety factor is already introduced by assessing the worst-case label use patterns. The protectiveness of the single product risk assessment for the realistic situation with multiple products treatment needs to be further investigated considering these conclusions.

TU025
efam: automated modeling software for environmental risk assessment
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For the registration of plant protection products the agrochemical industry needs to provide environmental risk assessments for active substances and related products. To conduct these risk assessments, obligatory and officially provided third-party computer models are used in many countries worldwide. The computer models are used to obtain an estimate of the amount of substance that will enter the environment (Predicted Environmental Concentrations, PEC). PECs are calculated for different environmental compartments by different simulation models. Hence, in an environmental risk assessment several different models need to be set-up, parametrized, or run separately. This decentralized working procedure unfortunately implies significant drawbacks. The main issues are: replicated entry of identical input data, great extent of manual work, high quality assurance effort, challenging data management due to scattered information of the input/output files, and time-consuming workflow documentation. To make the whole procedure more efficient, knoell developed the modular built and extendible software package efam (environmental fate automated modeling) to facilitate automated modeling and reporting. The aim was to create a software application that can drive regulatory necessary computer models and evaluate the results from one single platform in an automated manner. The software is developed in a modular structure allowing for integration of individual modeling tools. This structure enables flexible adaptation at the modular-level, e.g. when new models are released, or new model versions are made available and need to be applied. Using efam, model calculations do no longer need to be carried out on desktop computers, but can be performed on a more powerful and remote server located in a secured data center. The software can help automating and optimizing the workflow for individuals or groups of people dealing with environmental fate and exposure assessment. It is developed to automate model parameterization and model simulations, extract the results and transfer the output to formatted tables suitable for dossier/report incorporation. The development is of interest, not only in terms of speeding up modeling and report generation, but also it will optimize the organization of data, reduce the occurrence of manual input errors and reduce the effort required for quality control. Simplified data exchange with relevant stakeholders is currently under discussion as further field of application.

TU026
Ecological relevance of the Biota Quality Standards
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Biota Quality Standards were set by the European Commission with the goal to protect top predators and humans from secondary poisoning by chemical accumulation in the food chain. In reality, a vast exceedance of these standards for a number of components is observed, without an eminent decrease in the ecological water quality. In the present study, the relationship between accumulated concentrations in muscle tissue of European eel (*Anguilla anguilla*) and European perch (*Perca fluviatilis*) and the ecological water quality was investigated. Fishwere collected from 33 different sampling locations throughout Flanders (Belgium). Accumulated concentrations of HCB, Hg, PBDEs, HBCD, PFOS, heptachlorepoxide, dioxins and PCBs were measured. Additionally, 2 PAHs were measured in translocated quagga mussels (*Dreissena bugensis*). Ecological water quality was assessed using the Index of Biological Integrity (IBI), applying fish community, and the Multimetric Macroinvertebrate index Flanders (MMIF). The main goal of the study was to define a threshold value for which accumulated concentrations reflect a decrease in ecological water quality. These results could have an important impact on the revision and fine-tuning of current Biota Quality Standards.

TU027
Freundlich sorption-desorption isotherms of hexazinone in the bonechar-amended soil using a bath equilibrium method
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Hexazinone [3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] belong to the chemical group of the triazinone, and is selective herbicides inhibitors of photosystem II, used in pre- and post-emergence of annual grasses and broadleaf weeds, and can be easily leached to groundwater sources. Bonechar (BC), as well as biochar have been shown to strongly sorb organic compounds and could be used as an adsorbent to increase herbicides sorption and decrease leaching, although its application form is still little known. The aim of this research was to assess the absorbent effect of a cow BC on the sorption-desorption of hexazinone in Ultisol-Typic Hapludalf (sandy loam texture) using a bath equilibrium method. The BC was added to soil at 0 (control – unamended) and 5% (w w-1) ratio, corresponding 60 t ha-1. Five solutions (0.63, 1.25, 1.88, 2.50, and 3.13 mg L-1) were prepared of [triazine-6-14C] hexazinone (radiochemical purity 99.7%, specific activity 3.14 MBq mg−1). Sorption-desorption isotherms were calculated from the Freundlich model. The Kf values calculated for the hexazinone sorption on the unamended and BC-amended soil were 0.12 and 20.76 μmol (1-1/n) L1/n kg-1, respectively. Desorption on the unamended and BC-amended soil was 6.58 and 0.55%, respectively. The 1/n(desorption) are greater than 1/n(sorption), suggesting that the hexazinone sorption by all treatments was reversible. H values ranged from 1.08 to 1.59 for hexazinone on all treatments. The results indicate that addition of BC promotes the high sorption and low desorption of hexazinone in the surface soil. We assume it is possible to minimize the pollution of soil-applied herbicide simultaneously as the BC, and then hexazinone can remain in the soil for a long time. However, this carbonaceous material may influence negatively the herbicide efficacy on weed control, which is worthy of further investigations, especially in field conditions.

Can Regulatory Risk Assessment Protect Wildlife? (P)

TU028
Birds risk assessment scheme vs real life; Polish experience
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According to EFSA guidance *Risk Assessment for Birds and Mammals* (2009), several species of birds are included. Step by step evaluation traverses from ‘indicator’ to ‘focal’ species, where latter one must be representative of real species occurring in particular crop and its growth stage. In this approach, a positive outcome of evaluation indicates low risk for the bird’s population. But is it safe for them in reality? From year 2000, population of bird’s species in Poland is estimated as a part of national multiannual state environmental monitoring programme. The latest Chief Inspectorate of Environmental Protection report: *Monitoring of Birds of Poland (2015-2018)* investigated state changes in common birds population in the agricultural landscape over the course of last 18 years. All collected data served to establish the Farmland Bird Index (FBI). Based on robust information provided by the national programme *Monitoring of Birds of Poland* and harmonized standard risk assessment scheme (including focal species) compilation and comparison is presented.

TU029
Accuracy of GPS-tag tracking for wildlife risk assessment relevant field scenarios - the current situation
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The automatic acquisition of detailed animal movement data with GPS-tags is now a common scientific method and is still evolving. However, the improvement of data accuracy is not as much in focus as the drive to increase tag lifespan or decrease tag weight in order to monitor smaller species. Moreover, the main scientific focus is on the acquisition of long-term movement data (e.g. daily or monthly) to put animal movement behaviour in context, for example with respect to biotic and abiotic parameters. However, the main requirements of data intended to support risk assessments conducted for the purpose of pesticide registration are the continuous minute-by-minute recording of the exact position and movements of tagged individuals. GPS-tags are currently available for medium-sized species such as hares and these tags are able to record highly detailed spatio-temporal movement data over several days without any undue disturbance to the tagged animal. However, detailed data require short intervals between recording events effectively limiting the life of the tag’s battery. The GPS-tag method for recording animal movements can also be used in pesticide risk assessment field studies, for example to determine so-called PT values (i.e. estimations of the time tagged animals spend in different crops) or verify exposure in treated crop fields. However, one of the main concerns associated with this method is its accuracy and this can be a crucial requirement when tracking species such as lagomorphs moving along field margins and borders. Depending on the risk scenario to be covered, the behaviour of the species under investigation and the distribution of the crop of concern within an agricultural landscape, the issue of accuracy can be of greater or lesser importance. In order to assess the reliability of GPS-tag data sets for use in regulatory processes, GPS-tags need to be evaluated and their advantages/disadvantages discussed and compared to more commonly applied methods. We therefore present GPS-tag data for medium-sized mammals showing the accuracy of recorded locations under different field scenarios (including measurements of activity). Our results indicate that species ecology, landscape structure and other factors need to be considered when deciding whether currently available GPS tracking systems can provide reliable PT estimates for pesticide risk assessment as required by the current EFSA wildlife guideline.

TU030
Bats, birds & shrews in environmental risk assessment
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Bats are not specifically considered in the current pesticide risk assessment guidance for birds and mammals in the European Union, which evaluates the risk to birds and to mammals such as rodents, shrews and lagomorphs based on dietary exposure. The implicit assumption seems to have been that all mammals, including rather unique groups such as bats, are protected by the current avian or mammalian risk assessment scheme. However, the biology of bats differs in various aspects to these groups. It is therefore reasonable to question whether bats should potentially be considered as an additional species group in terrestrial vertebrate risk assessments performed in the EU. Therefore, we have conducted a review of the publically available literature and have compiled a list of relevant European bat species that potentially are present in the agricultural landscape. The major and minor crops of importance as foraging habitats have also been established, whilst considering foraging techniques for added context. As food intake is considered the main route of bat exposure to pesticides, information on daily energy expenditure and subsequent food intake has also been gathered from the literature to determine appropriate food intake rates. These data have been compared to the current insectivorous food intake rates for birds and mammals given in the current EFSA Bird and Mammal Guidance Document (2009). Toxicological endpoints for bats gathered from publications on the impact of pesticides were identified regarding their suitability to be used in risk assessments. Comparisons are made between endpoints for bats and commonly agreed toxicological endpoints of pesticides from laboratory studies with rats and mice, and the results are discussed. With this information, environmental risk assessments based on the same prerequisites following main concepts of the EFSA Bird and Mammal Guidance Document were conducted for insectivorous birds and shrews and for representative bat species. A comparison of these scenarios will be made and the outcome will be discussed, in order to evaluate whether the risk of pesticides to bats is sufficiently assessed and covered by the currently performed risk assessment scheme for birds and ground-dwelling mammals.

TU031

Can regulatory pesticide risk assessment scheme protect insectivorous bats?

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The chiropterofauna is an important component of the ecosystem in various types of landscape. It has been proven that 28 species of insectivorous bats of two main families (*Rhinolophidae* and *Vespertilionidae*) mostly occur in the Slovakia. In our literature review we had identified two critical periods, when bats may be under certain scenario exposed to residues of applied pesticides. First one is reproduction season of bats which lasts in Slovakia from May to July. This is very sensitive period for the bats, because there must be abundant prey available near the roost of summer colony. Pesticides should be applied to crops with little or no hazard to honeybees. That is why insecticides with_longer term toxic effects or tank mixes (synergistic toxic effects to insects, e.g. pyrethroids with fungicides) require special protective measures. One of the most commonly used restrictions in such cases is late evening/night application. If insecticide with prolonged residual toxicity is applied in the evening/night, it can not only harm insects but secondary also the insectivorous bats. The time before hibernation may be considered as second critical period for bats. During autumn bats must build up energy reserves for hibernation, it's a period of intense hunting. Because pesticides are not applied in such quantity in comparison with late spring and summer applications, we do not expect acute effects. Based on Guidance of EFSA (2009) proposals for selection of bird and mammal species as focal species we identified total of five bat species which at least partly fulfil set criteria. The Lesser Mouse-eared Bat (*Myotis blythii*) and the Greater Mouse-eared Bat (*Myotis myotis*) are specialized in hunting insects crawling on the ground. The Noctule Bat (*Nyctalus noctula*) and the Serotine (*Eptesicus serotinus*) are quite commonly observed in Central Europe, hunting insects by rapid flights along vegetation edges or in open areas; they also pick prey directly from the ground or from the canopy of trees. The last one selected bat is the Common Pipistrelle Bat (*Pipistrellus pipistrellus*) weighing only 3.8 to 7.0g, i. e. rapid energy exchange makes it ideal focal bat species with numerous small flying insects found in its diet. Potential harmful effect to bats needs to be reflected more detailed in future research and maybe the result will be that some pesticide with extended residual toxicity can require additional application restrictions to those indicated on the label.

Understanding the Complexity of the Natural Aquatic Environment Facing Global Changes (P)

TU032

Biomarker approach to assess the toxicity of S-metolachlor and Terbuthylazine in the benthic clam Scrobicularia plana

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The overexploitation of the farmlands in the Mediterranean region contributes to an overuse of herbicides that have the potential to pollute adjacent aquatic systems. The Mondego estuary, located near the city of Figueira da Foz (Portugal), is surrounded by farmland, namely rice and corn crops. Primextra® Gold TZ is the most-used herbicide in this area, being constituted by two main active ingredients, S-metolachlor (SMOC) and terbuthylazine (TBA) that are toxic for aquatic environments. *Scrobicularia plana* was selected to carry out bioassays because of its ability to filter pollutants and its key role in estuarine functioning. Organisms were exposed to a range of concentrations of each chemical, individually. *S. plana* showed higher sensitivity to SMOC (big - LC_{50}=40.702 mg/L; small - LC_{50}=41.517mg/L) than to TBA (big - LC_{50}=118.590mg/L; small - LC_{50} =108.418 mg/L),being big size class, generally, the most tolerant. The sensitiveness of organisms to pollutants' exposure is associated to its ability to cope with oxidative stress. Thus, antioxidant enzymes (glutathione peroxidase (tGPx), glutathione reductase (GRed) and glutathione-S-transferase (GST)), besides the quantification of TBARS were analysed through spectrophotometry-based methods.To both size classes, GST activated under SMOC exposure, surpassing the levels of activity from controls and from the field. GRed showed a quite specific response to TBA, being activated progressively through growing concentrations of chemical, especially the activities of big size organisms that surpass the levels from the field.The present work highlights GRed and GST are suitable biomarkers of the toxicity to the studied substances in *S. plana*. GST demonstrated to be more accurate biomarker for SMOC toxicity as it rises its activity under lower concentrations than of TBA. Thus, the search for biomarkers is crucial to the implementation of measures to rehabilitate polluted aquatic environments before the damages turn irreversible. This study was supported by Fundação para a Ciência e a Tecnologia (FCT) through the strategic projects UID/AMB/50017/2013 granted to CESAM and UID/MAR/04292/2013 granted to MARE. FCT funded A. F. Mesquita and A.M.M. Gonçalves, (SFRH/BD/139831/2018 and SFRH/BPD/97210/2013),co-funded by the Human Potential Operational Programme (National Strategic Reference Framework 2007–2013), European Social Fund (EU), and the program POPH/FSE.

TU033

Combined biochemical impacts of pollutants and temperature on two freshwater primary producers

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The hazardous impacts of broadly used pesticides on non-target species has been widely documented. Aquatic systems are particularly endangered, as substances reach the systems by water run-off, endangering its community and structure. The effect of Copper Sulphate and Tebuconazole was assessed for *Lemna minor* and *Raphidocelis subcapitata* exposed to combined bioassays of a contaminant and a temperature (20ºC and 25ºC), regarding impacts in the species' fatty acid (FA) and carbohydrate (CHO) profiles. CuSO4 effects depended on the temperature for both FA and CHO profiles. At 20?C, species presented the lowest FA content at a contaminant concentration of 1.20mg/l for *R. subcapitata* and 0.40mg/l for *L. minor*; with the increase of contaminant concentration, CHO content of *L. minor* increased, but decreased in *R. subcapitata*. At 25?C, the lowest FA content occurred at a concentration of 1.55mg/l in *R. subcapitata* and 1.76mg/l in *L. minor*, while the lowest CHO content occurred at the highest contaminant concentration in both species (2.20mg/l for *R. subcapitata* and 1.93mg/l for *L. minor*). The effect of Tebuconazole differed among species: in *L. minor*, the lowest FA content occurred at a concentration of 0.10mg/l at 20ºC and 0.12 mg/l at 25ºC, while in *R. subcapitata* FA content decreased with the raise of contaminant concentration, regardless the temperature. Tebuconazole induced an increase in CHO content at 20?C in *L. minor*, while resulting in a decrease in CHO content at 25?C in *R. subcapitata*. Primary producers, such as the species referred, are major sources of energy and essential long-chain polyunsaturated fatty acids in trophic chains, with animals not capable to produce these compounds at relevant rates. This study highlights the harmful effects of pesticides on primary producers and thus, affecting the whole trophic web, stressing the need for preventing major impacts on ecosystems by chemicals' usage and adapting pest control to ecosystem-sound practices.This study was supported by FCT through the strategic projects UID/AMB/50017/2013 granted to CESAM and UID/MAR/04292/2013 granted to MARE. FCT funded C. P. Rocha, T. Vidal, A. P. Cuco, N. Abrantes, A. M. M. Gonçalves, C. Nunes (SFRH/BD/140922/2018; SFRH/BPD/94562/2013; DPA 17-781; IF/01198/2014; SFRH/BPD/97210/2013; SFRH/BPD/100627/2014). The research was partially supported through the project Ref. POCI-01-0145-FEDER-022127.

TU034

Do toxins offer a competitive advantage for cyanobacteria facing global changes?

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Aquatic ecosystems are complex and dynamic adaptive systems driven by multiple biotic and abiotic factors. Over the past three decades, the increasing anthropic pressures, which are exerted on natural aquatic environment have caused the multiplication of cyanobacterial blooms and enhanced the incidence of the proliferations within these ecosystems. Moreover, it was clearly demonstrated that global changes have a large influence not only on the multiplication of cyanobacterial blooms but also on the occurrence of blooms of toxin-producing cyanobacteria. In this context, it is important to understand cyanobacteria adaptation to global changes and in particular, the underlying mechanisms that could offer ecological advantages to toxin-producing genotypes, allowing them to dominate the cyanobacteria bloom. Hence, the influence of two abiotic factors (light and temperature) was evaluated on toxin producers and non-toxin producers. We hypothesized that cyanobacteria synthetizing toxins will be more resistant in light and temperature stress conditions thank to a possible role of toxins in photosynthesis and/or photoprotection. In order to test this hypothesis, four *Planktothrix* strains were selected: two microcystin (a potent cyanotoxin) and two non-microcystin producers. A 24-hour experiment composed with a 4-hour stress exposure followed by a recovery phase was performed. The cultures in triplicate exposed to high light intensity (150 μmol.m^{-2}.s^{-1}) or high temperature (33°C) were compared with controls (20°C, 6 μmol.m^{-2}.s^{-1}). The effects of stress were evaluated on the relative expression of genes involved in photosynthesis (*psaA, psbA, acpA, cpcA, fnr*), photoprotection (*ocp, frp, flv1, flv3, hlip*), temperature tolerance (*dnaK, groEL, groES, hslO, hspA, hspG*), oxidative stress regulation(*sodB, gor*), toxins synthesis (*mcy*) and other secondary metabolites synthesis (*mdn, aer, oci, apt, mic)*. Our results revealed that cyanobacteria have specific responses regarding the nature of the stress with impacts of genes involved in photosynthesis and photoprotection in light stress conditions, while the temperature stress impacted the heat shock proteins. Nevertheless, the data obtained do not confirm that microcystin plays a specific role in photoprotective mechanisms during the first hours of stress since photosynthetic capacities were more impacted for microcystin producers compared to non-microcystin producers.

TU035

How climate change enhances nanoparticle toxicity towards freshwater biofilms

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Aquatic ecosystems are threatened by multiple environmental stressors. Release of toxicants such as engineered nanoparticles (ENPs) into aquatic systems and their effects on these ecosystems remain poorly understood. This, combined with the acceleration of global climate warming (ENP + ΔTºC), could have significant consequences for aquatic life. This study focus on responses of fluvial biofilms, as key points of ENP entry into aquatic food webs, and examines the effects of combined stressors at community and ecosystem levels using an extended set of end-points as an integrated approach. To achieve this aim, an outdoor microcosm study using different temperatures (18ºC and 25ºC) was performed. After 4 weeks of biofilm colonization at both temperatures, ENP were added obtaining 4 treatments (x5 replicates each): (1) control , (2) 50 μg/L of PVP-coated AgNPs (50 nm), (3) 25 μg/L of Ag_2S NP (30 nm) aged from pristine AgNP as a stable form of silver, known to have lower dissolution potential, and (4) 50 μg/L of AgNO3 as a positive (Ag^+) control. Sampling was done at the beginning of ENP exposition (0h), and after 1, 3, 15 and 30 days. Functional and structural changes were assessed using a range of end-points: enzyme activities and related metabolites, quantification/identification of secreted biomolecules and resulting ENP-coronas, analysis of extracellular polymeric substances (EPS), metabolism, photosynthetic activity, algal biomass, ENP bioaccumulation, and confocal and taxonomic observations of biofilm community composition. Metabolism increased with increased temperature but reduced in the presence of AgNP and Ag^+. Surprisingly, under warm conditions Ag_2S decreased the photosynthetic activity and algal biomass like the Ag^+ control, while AgNP increased both. Increased water temperature also caused homogenisation of biofilm composition and structure, and combined with AgNP or Ag^+, an increase of dead cells and a decrease of biofilm EPS. These results point out that warmer water can enhance AgNP and Ag^+ potential toxicity as well as modifying the behaviour of "non-toxic" compound like Ag_2S. These biological changes contrast with the fact that chemical (metal) analyses could not detect Ag in the water (total dissolved Ag^+) as concentrations were below the limit of detection of ICP-MS. This reinforces the importance of biological assays as early warning indicators. Results from chemical analyses and the rest of biological assays will be presented in detail.

TU036

Damselfly larvae become more sensitive to the pesticide chlorpyrifos under a neglected aspect of global change: increased thermal variation

J. Verheyen, R. Stoks, KU Leuven / Biology

There is increasing concern that climate change may make organisms more sensitive to chemical pollution. Many pesticides are indeed more toxic at higher mean temperatures. Yet, we know next to nothing about the effect of another key component of climate change, the increase of daily temperature fluctuations (DTFs), on pesticide toxicity. Therefore, we tested the effect of the pesticide chlorpyrifos under different levels of DTF (constant = 0°C, low = 5°C (current maximum level) and high = 10°C (predicted maximum level under global warming)) around the same mean temperature on key life history and physiological variables of *Ischnura elegans* damselfly larvae in a common-garden experiment. Notably, chlorpyrifos did not cause mortality or reduced growth rate at the constant temperature (0°C DTF), yet increased mortality 6x and reduced growth rate with 122 % at low and 111 % at high DTF. This indicates that daily short-term exposures to higher temperatures are enough to considerably increase pesticide toxicity. Moreover, the here used CPF concentration would have been regarded a NOEC ('No Observed Effect Concentration') if we had only tested the species under the standard conditions of constant 20°C. These standard conditions are typically used in laboratory ecotoxicity testing, while at the current DTF (going up to 5°C in this region), this CPF concentration may cause considerable mortality. However, a further increase from 5° DTF to 10°C DTF may not result in a further increase of pesticide toxicity. Our study adds to the few others showing an increased toxicity of pesticides under DTF and our results highlight the biological importance of including daily temperature fluctuations in ecological risk assessment of pesticides and as an extra dimension in the climate-induced toxicant sensitivity concept.

TU037

Impact of agricultural run-off and climate warming on key organisms and ecosystem functions in shallow aquatic ecosystems

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Aquatic ecosystems are exposed to a multitude of stressors such as climate warming and agricultural run-off (ARO). This might cause severe disturbances up to the loss of ecosystem functions and services. Within the project CLIMSHIFT, we study the impact of these stressors on shallow aquatic systems by using micro- and mesocosms combined with modelling. Our objectives are to understand and reveal individual and interactive mechanisms of climate warming and ARO by using a combination of response variables at the individual over the community level up to major ecosystem functions. In two microcosm experiments, we tested the effect on competing primary producers in shallow aquatic systems: submerged aquatic plants, benthic and phytoplankton algae. We used a factorial design with 2 temperatures and 6 ARO levels. Experiment I used a concentration-response curve for a fixed ARO mixture, comprised of nitrates, copper, a herbicide, an insecticide and a fungicide. Experiment II used a defined low concentration of the same ARO, applied as single, double and triple dose either at once or pulsed. Exp_II tested microbial respiration in the biofilm and sediment as well as the degradation of leaf litter. In Exp_I, phytoplankton biomass increased with ARO treatment, and more strongly at higher temperature. Periphyton biomass increased first with increasing ARO concentration and then declined. Photosynthetic yield declined in both phytoplankton and periphyton with increasing ARO concentration. ARO only affected plant length in in one of three submerged macrophytes, and temperature caused an increase in plant length in the other two species, while ARO had no effect. In Exp_II, a pulsed addition of the ARO cocktail had in general less effect than the same amount of pollutants added in a single dose. In general, comparable effects for phytoplankton and periphyton were observed at the same concentration of ARO tested. A lower photosynthetic activity was measured in two of the macrophytes. Microbial respiration was much stronger at 22°C than at 26°C in both biofilm and sediment. Leaf litter degradation was significantly affected by both ARO and temperature. Ultimately, shifts in primary producers might lead to a regime shift in shallow aquatic ecosystems and to the loss of submerged aquatic plants. This will cause a loss in ecosystem functions and services and thereby a shift towards an inferiour ecological status.

TU038
Effect of organic pollutants on Daphnia magna
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Effect of organic pollutants on *Daphnia magna* Asmerom Seyoum, Ajay Pradhan, Jana Jass, Per-Erik Olsson **Biology, the Life Science Center, School of Science and Technology, Örebro University, Örebro, Sweden**
<strong>Abstract Detection of organic contaminants in the aquatic environment at low concentrations, has raised concerns for animal and human health. Phthalates and perfluorinated alkylated substances (PFAS) are among the most frequently detected persistent organic pollutants in the environment. Phthalates are mainly used as plasticizers to increase durability, resistivity and flexibility of plastic material. However, they are not chemically bonded to PVC and they may leach out and enter into the environment. Similarly, PFAS have many industrial applications due to their unique surfactant properties as well as their remarkable stability that results from high energy carbon-fluorine bonds. The increased use of these persistent compounds has resulted in contamination of the aquatic ecosystem and may threaten the aquatic life. We used *Daphnia magna* as a model organism to study effects of the most commonly detected phthalates i.e. di (2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP) and dibutyl phthalate (DBP) as well as the widely used PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). *D. magna* were exposed to 1 and 10 μM phthalate and 1, 10 and 25 μM PFAS to investigate the chronic effects on hatching, development, reproduction, fat metabolism and lifespan. Exposures to phthalate and PFAS didn't result in acute lethality. However, preliminary data indicate that PFOS at 10 and 25 μM results in hatching delay of *D. magna* ephippia. Analysis of fat metabolism indicates that DEHP, DEP and DBP increase fat accumulation in Daphnia, and gene expression analysis following phthalate exposure in *D. magna* suggest that fatty acid accumulation is due to inhibition of enzymes involved in fatty acid uptake and catabolism. DEP and DBP did not alter the reproduction output, but surprisingly DEHP increased the reproductive output. PFAS on the other hand, decreased the reproductive output. Phthalate and PFOS exposure of *D. magna* led to decreased body length and lifespan. The results suggests that chronic exposure of *D. magna* to phthalates and PFAS at sublethal concentrations result in toxic effects. *Keywords*: Plasticizer, PFAS, Toxicity, Gene expression \n

TU039
Biodiversity, water quality and environment contaminants of the Bay of Bengal
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The Bay of Bengal is located at the northeastern end of the Indian Ocean and has an area of 2,172,000 km^2. Several major rivers run into the Bay of Bengal. The coastal areas of Bangladesh (along the Bay of Bengal) include 60% of the Sundarbans, the mangrove forest of the Ganges/Brahmaputra delta in the West and areas with coral reefs in the Cox's Bazar provinces in the East. These areas are economically important because of fishing and biodiversity due to rich source of flora and fauna. In order to determine biodiversity, water quality parameters and environmental contaminants, 110 water samples were collected from Mongla, Kuakata sea beach and Saint Martin area. pH of samples from Kuakata were ranged from 7.58 - 8.77 with an average of 8.83 ± 2 which indicates lower ion presence in the water samples. Total Organic Carbon (TOC) is an indirect is an indirect measure of organic molecules present in water bodies and measured as carbon which was analyzed for the collected samples by TOC analyzer and found in the range of 0.78- 1.78 mg/L with an average 1.24 mg/L. Concentration of NO_3^-, SO_4^{2-} and PO_4^{3-} were determined by ion chromatography and only SO_4^{2-} was in a very low concentration. Dissolved Oxygen (DO) and (BOD) of the water samples were determined by multi parameter analyzer. Average DO and BOD were 8.33 ± 1.82 and 0.31 ± 0.03 mg/L indicating that the water system were well oxygenated and good quality with low pollution. Water samples from seven different sources were analyzed for 5 metals such as Pb, Cd, Mn, Hg and As using Atomic Absorption Spectroscopy (AAS) and the contamination of metal elements were found in a very insignificant levels (Pb < 0.2 mg/L, Cd < 0.01 mg/L, Mn < 0.02 mg/L, Hg < 0.001 mg/L and As < 0.005 mg/L). Two seaweeds were collected from Saint Martin and were investigated for chemical diversity and will be reported in the presentation.

TU040
Ecotoxicity assessment of post-fire runoff on freshwater aquatic species
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Wildfire is a major disturbance of forests worldwide, posing an important threat to life, human goods and natural resources, not only on burnt areas, but also on adjacent aquatic ecosystems. Indeed, the environmental impacts of wildfires promote significant effects to the structure and functioning of the different ecosystem compartments. Wildfires have an important role in contaminants production and mobilization, such as polycyclic aromatic hydrocarbons (PAHs) and metals. These elements, mostly bound to ashes, can reach downstream water bodies by post-fire runoff, which can be toxic to aquatic biota. These detrimental off-site effects are particularly notorious during the initial post-fire period, although not necessary limited to it. However, the potential toxic outcomes of the input of such contaminants across the aquatic biota have received, so far, little research attention. This knowledge gap was addressed here through laboratory assays in which three aquatic species from distinct trophic levels: the algae (*Raphidocelis subcapitata*), the macrophyte (*Lemna minor*) and the invertebrate (Daphnia magna), were exposed to different dilutions of post-fire runoff and surface downstream water. The runoff was collected in a burnt and unburnt eucalypt stand as well as surface water from the burnt catchment outlet. The samples were collected during the first rainfall events after the wildfire, twelve and eighteen months later. We assessed the chemical composition in terms of PAHs and metals and effects of those runoffs on: (1) growth inhibition of *R. subcapitata* and *L. minor*; and (2) immobilization of *D. magna*. The chemical assessment proved the existing concern of wildfires impacts on aquatic systems, as a diffuse source of PAHs and metals. The results allowed discriminate the off-site effects of wildfires on distinct aquatic species. In general, unburnt surface runoff revealed to be less toxic to the producers, while the primary consumer was not significantly affected in any of the tested water samples. Moreover, it allowed to determinate the threshold concentrations of PAHs and metals that can be considered at low/high risk for aquatic biota. It was also demonstrated that detrimental off-site effects of wildfires are not limited to the immediately post-fire situation. This study highlights the need to additional research to understand the complexity of the potentially deleterious ecological effects of wildfires on aquatic communities.

TU041
Forest fires as potential triggers for mobilisation of major and trace elements (metals) to the aquatic system
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The increasing occurrence of wildfires and their overall impacts on the environment highlight the need for an effective post-fire forest management, from ecological as well as socio-economic point of view. Wildfires and subsequent rainfall can play an important role in the environmental (re)mobilisation of major and trace elements (metals), which could be leached into the soil profile or transported downslope by surface runoff, impacting both ground water and surface water quality. In particular, fire-induced inputs of these elements into the environment are relevant due to their toxicity, environmental persistence and tendency to bioaccumulate and bioamplify along the trophic chain. However, the impacts of wildfires followed by rainfall on metals redistribution on water bodies are poorly documented. In this context, the present study evaluates the impacts of a wildfire on surface runoff and nearby water downstream the burnt area by metals (V, Mn, Co, Ni, Cu, As, Cd and Pb) in north-central Portugal. Surface runoff from burnt, unburnt and water downstream an eucalypt area were collected over five campaigns during 18 months, to evaluated the influence of precipitation and time-since-fire on the levels of metals loads as well as the impact attenuation. The chemical assessment revealed concerning levels of some metals in burnt runoff and stream water samples. In general, the burnt surface runoff presented the highest levels metals, while the concentrations in the stream water samples were higher than the unburnt surface runoff. The behavior of the major and trace elements studied with time-since-fire revealed differences among them. The research results have contributed to improve and clarify the role of wildfires and rainfall on the (re)mobilisation of metals, pointing out that wildfires can act as a potential source of environmental contamination of aquatic systems. Monitoring source water downstream of burned watersheds allow water managers to minimize adverse water-quality effects, such as by temporarily diverting compromised water or changing source water. Understanding the effects that wildfire have on water quality is essential for ecosystem management and risk assessment.

TU042
Linking individual fitness to population changes of the endobenthic worm Hediste disversicolor: A temporal case study in the multi-polluted Seine (France) estuary
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Bruneau, A. Chatel, Catholic University of the West / UBL, Mer Molécules Santé; C. Mouneyrac, Université Catholique de lOuest / UBL, Mer Molécules Santé
The Seine estuary plays an important ecological and socio-economic role but is also the most stressed estuary in France. The northern mudflat of the Seine estuary is continuously subjected for long time to complex environmental stressors, such as chemical pollution and physical modifications such as harbor construction (*e.g.* the construction of "Port 2000" from 2001 to 2005). To assess the potential effect of those stressors (both natural and anthropogenic), in the last decade, monitoring programs focused on the integrative quality assessment of this vulnerable natural habitat using the endobenthic worm *Hediste diversicolor* as sentinel species (*e.g.* The National French Program of Ecotoxicology "PNETOX"). It seems now a necessity to collect and compare data from anterior programs in order to evaluate the environmental status of this site as well as the relevance of indigenous populations as an appropriate environmental quality tools. In this context, the aim of the present study was to compare potential links between individual fitness (growth, allometric relationships, energy reserves, reproduction status) and population changes (density, biomass and populations dynamics endpoints) in worms (*H. diversicolor*) seasonally collected during "the Port 2000" construction (2002-2004) and fourteen years after (2018). The modal decomposition of size distribution frequencies revealed that during the first period (2002-2004), *H. diversicolor* populations were characterized by a unique cohort and consequently, a single-modal recruitment of juvenile worms. In contrast during 2018, population analysis demonstrated the existence of two simultaneous cohorts and a bi-modal recruitment. Worms density was generally lower in 2003 and 2004 as compared to current (2018) density whereas an inverse pattern was observed for biomass. Individual growth parameters (L3 and weight) did not show any significant differences between sampling periods. Allometric relation-ships and energy reserves comparisons between the two studied periods are actually in progress. Preliminary results, issuing from linking individual fitness to populations changes could be indicate an amelioration of the environmental quality of the Seine estuary northern mudflat after the combined stress episode (2002-2004). The overall results demonstrate the relevance of using *H. diversicolor* population's endpoints for the environmental assessment of estuarine ecosystems in a combined stressors context.

TU043
Nutrient pollution determines membrane lipid profile of coral responded to ambient warming
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Thermal stress causes mass coral bleaching and mortality. Nutrient pollution altering coral physiology increases the risk of health. Membrane lipids constitute the basic structural element to create cell a dynamic structure according to the circumstance. Characterizing the lipid profile of coral responded to environmental factors could give an insight into the mechanism of effect. Glycerophosphocholine profiling of the coral Seriatopora caliendrum responded to warming conditions (28–32?) under different nitrate loadings (0.05–0.5 mg/L as N) was performed using a lipidomic methodology. The symbiotic alga-related physiological factors of the coral were altered by increased nitrate, warming stimulation and the interaction. Warming-induced lipid variations in the coral were well modeled based on the incubated temperature according to the nitrate loading. Based on the physicochemical properties, the commonly changed lipids logically indicated a membrane accommodation relating to ambient warming and the induced photophysiological change in the coral. The changed lipids specific to the exposure loading of nitrate suggested a cause increasing the health risk of the coral. Although the increased nitrate alone did not induce a pronounced change in lipid metabolism of the coral, in conclusion, the effect was identified while acts with a warming condition.

TU044
ECORISK2050: Effects of global change on the emission, fate, effects and risks of chemicals in aquatic ecosystems.
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By 2050, the world population will reach nine billion people and three quarters of the global population will live in cities. The development path to 2050 will be marked by shifts in land-use and weather patterns, and by changes in the way water and food resources are obtained and managed all over the world. These global changes (GCs) will affect the emissions, environmental transport pathways and fate of chemicals, and thus affect the exposure of the natural environment to chemicals. Future changes may also alter the sensitivity of ecosystems to chemical exposure. Therefore, the ECORISK2050 project brings together a world leading and interdisciplinary consortium of universities, research institutes, industry and regulatory and governmental authorities to deliver a cohort of Early Stage Researchers (ESRs). The coupled training goals and research objectives of the project are: (1) to assess how the inputs of chemicals from agriculture and urban environments and their fate and transport are affected by different environmental conditions, including those of specific EU regions, and how this will change under GC scenarios in order to assess the likely increase in chemical risks to human and ecosystem health; (2) to identify potential adaptation and mitigation strategies that can be implemented in the short and medium term, to abate unacceptable changes in risks, and use the GC scenarios to propose robust implementation pathways, and (3) to develop a set of tools for use by industry and policy makers, that allow the impacts of a range of GC-related drivers on chemicals risks to be assessed and managed. The project will deliver the next generation of scientists, consultants and industry and governmental decision-makers who have the knowledge and skill sets required to address the changing pressures that chemicals emitted by agricultural and urban activities pose to aquatic systems on the path to 2050 and beyond.

TU045
Anionic Herbicides: Using IAM chromatography to determine if acute effect concentrations to aquatic organisms are below baseline-toxicity
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Many herbicides are acidic compounds, the most notorious one probably 2,4-D (pKa 2.7, logD7.4 -0.8, soil half life 34d). Several of these herbicides have relatively long aerobic half-lifes in soil, extending the PMT limit of >40 days. Due to the low pK_a, these herbicides are present predominantly in their anionic form in most environmental systems. This makes these compounds highly mobile. Of course, herbicides are meant to be specifically toxic to certain types of plants they are meant to control. As such, many anionic herbicides fit the criteria of PMT compounds. For the strict regulation dossiers of pesticides, these compounds are widely tested for toxicity to non-target species, particularly covering several taxa of aquatic organisms. It makes sense that many herbicides could be toxic to algae, but to what extent are they toxic to invertebrates and fish? All organic chemicals, including ionogenic compounds, have a baseline toxicity that is induced at a critical accumulate level in cell membranes, called the critical membrane burden (CMB). Ideally, a herbicide is specifically toxic to certain plant species, and exert no specific toxicity to any non-target organism other than the baseline toxicity any organic chemical presents (alone or in a mixture). In this study, we evaluated the affinity of 10 anionic herbicides to bind to cell membranes (K_{MW}) using a chromatographic column coated with immobilized phospholipids (IAM-HPLC). Using the obtained K_{MW}, the narcotic effect concentration ($LC_{50,narc}$) was predicted based on a critical narcotic cell membrane burden of 50-200 mmol/kg. The $logK_{MW,IAM}$ values were orders of magnitude higher than logD values. The predicted baseline $LC_{50,narc}$ values closely correspond for many herbicides to acute LC_{50} for daphnids and fish, while algae are effected by 1-4 orders of magnitude lower. This distinguishes the herbicide specificity. Exception is dinoseb, a known uncoupler of oxidative phosphorylation, which shows even higher toxicity to fish that to algae. This evaluation may inform regulators on the non-specificity of anionic herbicides to non-target organisms beyond a logD approach. It additionally relates to the potential pressure of the compound to the total toxicant stress of chemical mixtures in contaminated environments.

Towards a Sustainable Development of River-Sea Systems (RSS) and Coastal Areas (P)

TU046
MONITOOL: New tools for monitoring the chemical status in transitional and coastal waters under the Water Framework Directive
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Clinica e Molecolare
In highly dynamic systems, such as transitional and coastal waters, establishing their chemical status is challenging. MONITOOL is an exciting European project consisting of 16 Partners covering the Atlantic region from the Canary Islands to the Scottish Highlands and Islands, which aims to address this complex analytical challenge, responding to European Directive [1] demands for the assessment of the chemical status of transitional and coastal waters. Diffusive Gradient in Thin Films (DGT), and passive samplers (PS), in general, are already widely used in investigative monitoring and there is an increasing interest in their use for the environmental assessment of water bodies, within European policies requirements. The main barrier hindering the regulatory acceptance of PS for compliance checking is the lack of appropriate Environmental Quality Standards (EQS). EQSs for metals are defined in the dissolved fraction, preventing the use of DGT-labile concentrations for the establishment of the chemical status of water bodies. The MONITOOL Project aims to define suitable EQS to allow for the use DGT devices [2] for the monitoring of these priority metals in a regulatory context. DGT design allows for the continuous accumulation of metals *in situ*, and subsequent quantitation via methods such as ICP-MS. While many of the chemical aspects of the devices have been well studied [3], effects of environmental physicochemical parameters on the functionality of the devices has not been examined in detail. Five-day deployments of DGT devices, alongside spot sampling and physicochemical parameter measurement, will be conducted in both wet and dry seasons in coastal and transitional waters of the North Atlantic coast. The first sampling campaigns were performed during winter 2017/2018 in 4 selected sites (transitional and coastal sites) in each consortium region (8 regions). All partners followed the same protocol for sampling and analysis to minimize the operational variability. Priority metals (Cd, Ni, Pb) and other specific metals (Al, Ag, Cu, Cr, Co, Fe, Mn, Zn) were analysed in waters and in the DGT resins. Statistical analysis is being applied to study relationships between metal concentrations in DGT and in spot water samples. Suitable EQS for DGTs will be calculated on basis the statistical relations obtained previously. This will permit a better implementation of the Water Framework Directive in variable systems like transitional and coastal waters.

TU047
The exposure of glyphosate and copper affect growth and reproduction on Daphnia exilis: a study transgenerational
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Currently, contamination of waters with glyphosate and copper is a worldwide problem; this is originated from agricultural, mining, and industrial activities. Although glyphosate is classified as an herbicide of reduced toxicity, several studies have demonstrated the occurrence of this pesticide at environmentally relevant concentrations, and toxic effects to aquatic biota have also been documented. Copper is an essential micronutrient at low concentrations; however, at high levels, it becomes toxic; this metal is also used as a biocide. Most chronic toxicological test guidelines only evaluate the toxic effects within one generation; however, negative effects could appear across generations, mainly when different toxics act jointly. We aimed to determine the transgenerational effects produced by mixtures of glyphosate and copper on the American cladoceran *Daphnia exilis*; measured endpoints were population growth, fecundity, metabolic biomarkers, and the size of the progeny. LC_{50}'s of both toxicants were determined. In the chronic toxicity studies *D. exilis* was exposed to the following mixtures of glyphosate (mg L^{-1}) and copper (mg L^{-1}): $LC_{0.05}$ (1.04 and 2.45); $LC_{0.5}$ (3.09 and 1.24); LC_5 (3.83 and 1.45) and LC_{10} (4.31 and 1.57), respectively. Experiments were performed during 21 d for each generation, at 25°C, 16:08 photoperiod, feeding with *Pseudokirchneriella subcapitata* ($8x10^5$ cells mL^{-1}). Survivorship, accumulated progeny and the number of clutches in the parental generation (F_0) were significantly higher than the values observed in F_1. Age of first reproduction was significantly different between F_0 and F_1 in the combinations $LC_{0.05}$ and $LC_{0.5}$. The concentration of lipids in *D. exilis* exposed to the mixture of glyphosate and copper in F_0 and F_1 was not significantly different in all the tested concentrations; nevertheless, carbohydrates content in F_1 was significantly increased respect to F_0 in $LC_{0.5}$ and LC_5. There was a reduction in the proteins content in F_1 compared to F_0, only in $LC_{0.5}$. The size of neonates varied among treatments and broods in F_0 and F_1; in F_0, some clutches were affected by the mixture of xenobiotics, especially in the $LC_{0.05}$ and LC_5 treatments. The mixture of glyphosate and copper significantly increased toxic effects on *D. exilis* in the F_1 generation, probably because in F_0 the cladocerans could develop resistance mechanism to tolerate toxicity, that influenced the response of progeny in the second generation.

TU048
Demographic transgenerational effects of glyphosate in the cladoceran Daphnia curvirostris
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Glyphosate is an herbicide extensively used around the world. Although it is supposed to be biodegraded in some weeks, the active ingredient, as well as the adjuvants and other compounds in the commercial formulations, can produce toxic effects in the hydrobionts, temporally and in their future generations, modifying the structure and function in the aquatic ecosystems. Cladocerans are an important component of the freshwater zooplankton, as it is a fundamental link between primary producers and secondary consumers. The toxic response to pollutants in the progeny of exposed parents is not normally investigated in routine toxicity tests, notwithstanding negative, transgenerational damages could be expected. This study was aimed to determine the transgenerational effects produced by the herbicide glyphosate, as the commercial formulation FAENA®, and as active ingredient (AI) in *D. curvirostris*, evaluating demographic responses. We determined the 48-h acute toxicity of FAENA and AI, then five sublethal concentrations were chosen ($LC_{0.01}$, $LC_{0.1}$, LC_1, LC_5, and LC_{10}) for the exposure of *D. curvirostris*, from neonates to adults, in two generations (F_0 and F_1), during 21 days; to determine the modification in the response to the toxicants in the F_1 generation, simultaneous replicates with individuals of the same clutch were not exposed to glyphosate (recovery phase, F_{1R}). Assessed demographic parameters were survivorship, total progeny, the age of first reproduction, number of clutches, and inter-clutch time; the size of neonates in each clutch and that of adults at the end of experiments were also measured. The LC_{50} in *D. curvirostris* was 5.85 mg L^{-1}; however, the LC_{50} for AI was higher than 100 mg L^{-1} and was not experimentally determined. Demographic parameters were significantly affected in F_0 when cladocerans were exposed to FAENA, but with AI there were no differences between F_0 and F_1. In F_1 and F_{1R}, a reduced effect in the demographic parameters was observed, possibly because the organisms acquired mechanisms of resistance, despite in F_{1R} the individuals were grown in fresh culture medium without toxicants. The results demonstrated transgenerational effects in *D. curvirostris*. Also, we confirmed that the toxicity of glyphosate is caused by the ingredients in the commercial product FAENA, although it is not possible to identify the joint effect of the active ingredient with the assessed endpoints.

TU049
Examining American alligators as sentinels of toxic trace element exposure
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Toxic trace element exposure occurs through anthropogenic release of naturally occurring elements such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg), making them ubiquitous across the globe. Detrimental exposures can occur through ingestion of contaminated food sources; thus, many nations have consumption advisories in place; however, not all communities abide. As a result, populations that include a large proportion of marine species in their diet, including subsistence hunting communities, are routinely exposed to concentrations above advisory limits. To effectively monitor environmentally-driven trace element concentrations, and consequently the potential exposure in humans, sentinel species are needed that are more accessible than the marine mammals currently used as proxies. Due to the unique environmental conditions of the southeastern Atlantic coast of the United States, accumulation of Hg in this region is greater than most other locations in the country. There are also point sources of As, Cd, and Pb in this region. Upper trophic level predators in this region exhibit elevated concentrations of Hg and other contaminants. In this study, concentrations of As, Cd, Pb, Hg and six other trace elements (Al, Ni, Cu, Zn, Se, Mo) were examined in American alligators (*Alligator mississippiensis*) from seven sites along the southeastern Atlantic coast. The observed concentrations for the four toxic elements (As, Cd, Pb, and Hg) were comparable to those reported in humans, with Hg spanning the broadest range. The similarity in As, Cd, Pb, and Hg concentrations between alligators and humans observed in this study underscores how alligators can serve as a useful sentinel species for toxic element exposure.

TU050
Toxicity of the Antifouling Biocide Sea-Nine 211 (DCOIT) to Neotropical Marine Invertebrates
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The antifouling biocide 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT), commercially known as Sea-Nine 211, came up to replace organotin based paints, which were banned in 2008. The toxicity of SeaNine has been recorded to various species of different trophic levels, but there are no studies evaluating its toxicity to neotropical species. The present study aims to evaluate the toxicity of SeaNine to the following neotropical species: *Perna perna* (Bivalvia), *Echinometra lucunter* (Echinodermata), and *Artemia salina* (Anostraca). The effects of DCOIT on the embryonic development of *E. lucunter* and *P. perna* were evaluated by exposing in vitro fertilized eggs to seriate dilutions of DCOIT (0.01 to 100 µg/L for *E.*

lucunter and 0.01 to 500μg/L for *P. perna).* For *A. salina* acute toxicity tests were conducted with nauplii, using concentrations between 10 and 1000μg/L. The respective $EC_{50\text{-}48h}$ obtained to *E. lucunter* and *P. perna* were 35.72 μg/L (17.71 – 71.02 μg/L) and 8.06 μg/L (6.96 – 9.36 μg/L), while the $EC_{10\text{-}48h}$ were 9.43 μg/L and 2.86 μg/L respectively. There was no statistically difference between the water and solvent control. In the test with *A. salina*, the $LC_{50\text{-}48h}$ and $LC_{10\text{-}48h}$ obtained were 173.9 μg/L (142 – 212.8 μg/L) and 94.96 μg/L respectively, both water and solvent control showed a survival of 100%. The LC and EC values obtained in this investigation are within the range of sensitivity found in literature for similar taxa representants. The DCOIT has been used as a substitute for TBT; however, the data presented in this work, supported by the literature, shows that both substances have a comparable toxicity (e.g. LC_{50} of TBT on the embryonic development of *P. lividus* = 37 μg/L). Thus, this study suggests that the environmentally friendly title labelled in the latter 1990's by the US.EPA may be not appropriate, and the SeaNine has the potential to cause ecological effects despite its rapid degradation in water.

TU051

Sub-lethal toxicity of chlorpyrifos to fresh water indicator mussel species Unio crassus Philipsson, 1788 (Bivalvia, Unionidae)

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Chlorpyrifos is a non-systemic organophosphorus insecticide, used in agriculture to control pests, and indoors to control mosquitoes and fire ants. The World Health Organization (WHO) classifies chlorpyrifos as a category 2 (moderately hazardous) pesticide. Chlorpyrifos is highly toxic to fish and aquatic invertebrates. The objective of this study was to determine the acute sub-lethal (24-h and 96-h) toxicity of chlorpyrifos to the fresh water mussel *Unio crassus* (Bivalvia). The mussels were hand collected from Karaçay, Sinop, Turkey and acclimatized for one month before the experiments. Stock chlorpyrifos solution was prepared in DMSO and diluted to provide exposure concentrations of 10 and 100 μg/L. Controls received only aerated municipal tap water. At termination of exposure times hemolymph was collected by diluting with 4% formaldehyde (1:1) and counted in Thoma counting chambers under a light microscope to calculate total hemocyte counts (THC/ml). After hemolymph sampling mussels were sacrificied under ice anestesia for histopathologic examination. Tissues were fixed in Davidson's solution and routine histologic procedures were performed. Mean values of the control group were 682667 and 534706 THC/ml for 24 and 96-h, respectively. The cell counts decreased with respect to controls during 24-h; but increased in 96-h. No differences were found between 10 and 100 μg/L exposure concentrations; neither between 24 and 96-h control and 100 μg/L THC/ml results ($P > 0.05$). However 24-h control and 10 μg/L groups were significantly different ($P < 0.01$). The 96-h experimental group data did not show normal distribution. Non-parametric Kruskal Wallis H test results showed significant differences ($P < 0.01$) between all groups for 24-h experimental duration. Degenerations were observed in the digestive gland tissues after 96 h exposure to 100 μg/L chlorpyrifos. Further research is needed to elucidate the molecular mechanisms of chlorpyrifos toxicity, comparison of in vitro and vivo studies in other marine or fresh water mussel species, together with non-target indicator organisms.

TU052

Sulfate losses from gypsum amended agricultural fields do not pose a threat to riverine species in the catchment of the Baltic Sea

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Eutrophication due to nutrient load from agriculture is a major problem in the Baltic Sea. Applying gypsum ($CaSO_4$? $2H_2O$) onto agricultural fields has a great potential to lower the phosphorus load. In 2016, a pilot study was started in south-western Finland, where gypsum was spread on 15 km^2 of clayey agricultural fields to reduce phosphorus losses to streams and rivers, and finally to the Baltic Sea. Gypsum is readily dissolved in the soil solution, which elevates the concentrations of calcium and sulfate in the downstream waters. Thus, the environmental effects of gypsum need to be assessed before large-scale applications can take place. Of these two gypsum ions, sulfate has reported to have a harmful effect on riverine biota, when present in high concentrations. An extensive risk assessment study was conducted to reveal the possible effects of the increased sulfate concentrations in a riverine ecosystem. The assessment consisted of an online monitoring of the sulfate levels and measuring the abundance of thick shelled river mussels (*Unio crassus*) and fish species. The laboratory studies included sulfate exposures measuring the growth of greater water moss (*Fontinalis antipyretica*), behavioural activity of adult *U. crassus* and acute survival test of *U. crassus* glochidia. The *in situ* experiments consisted of the measurements of algal biomass, behavioural activity of adult *U. crassus* and the survival and hatching of incubated brown trout (*Salmo trutta*)eggs. Some of the test species responded to the highest test concentrations of sulfate (1100-1200 mg l^{-1}). However, the sulfate concentrations observed in the river after the gypsum spreading did not have statistically significant effects on any of the test species or endpoints. In the river the abundance of some of the fish species declined after the gypsum spreading, but this was likely a result of natural environmental variation and fish stocking practices rather than gypsum spreading. The gypsum treatment of agricultural fields did not seem to pose a threat to riverine ecosystems at the study site. Yet, the sulfate levels of the rivers located in other gypsum spreading areas should be monitored to verify a safe sulfate concentration.

TU053

Sub-lethal toxicities of zinc pyrithione (ZPT), copper pyrithione (CPT) alone and in combination to the indicator mussel species Unio crassus Philipsson, 1788 (Bivalvia, Unionidae)

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Zinc pyrithione (ZPT) and copper pyrithione (CPT) are replacement antifouling booster biocides, and are emerging contaminants in marine and freshwater ecosystems and therefore may have ecotoxicological impacts on non-target organisms posing risks. The aim of this study was to determine the potential sub-lethal toxic effects of zinc pyrithione and copper pyrithione separately and in combination (ZCPT) to freshwater indicator mussel species, *Unio crassus* Philipsson, 1788 (Bivalvia, Unionidae, mean length = 54.9 mm). The mussels were hand collected from Karacay, Sinop, Turkey and adapted to laboratory conditions. Control and DMSO solvent control groups were compared with 10 $\mu g\,l^{-1}$ individual biocide and 5+5/10+10 $\mu g\,l^{-1}$ sub-lethalcombination exposure groups, keeping all tanks in the dark for photodegradation. Mussels were kept in 10 L aerated tap water and exposure durations were 48-h and 7-d; at the end of 48-h the animals received the same concentrations once more, after removing the initial media from the tanks. At the termination of exposure times, mussels were weighted, morphological measurements taken and then hemolymph collected by diluting with 4% formaldehyde (1:1) fixative. Haemocyte counts were made using a Thoma counting chamber under a light microscope, results reported as total haemocyte counts (THC/ml). Lipid peroxidation levels were measured as MDA (nmole/g tissue). Tissues were dissected, fixed in Davidson´s fixative, histopathological alterations were observed in digestive gland tissues. Degenerations were more severe at the copper pyrithione and the combination of the compounds groups. Mean THC/ml values differed significantly ($P < 0.001$) among all groups, for both exposure durations. MDA levels were lower than controls, but in the 7-d group increased above 48-h levels, the differences were not significant except for control-CPT 48-h and control-ZPT 7-d. GSH and protein levels were different during 7-d.

TU054

Ascidians as bio-indicators for pharmaceuticals in marine environments

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The use of pharmaceutically active compounds (PhACs) is increased every year in human and animal medicine worldwide. Their presence in the marine environment presents a threat to marine organisms and ecosystems. Measures of PhACs concentrations in marine waters and sediments are not sufficient to predict exposure concentration by marine organisms, as they provide only a snapshot in time. Additionally, it does not illustrate the harmful effects and the fate of these compounds within living organisms. Some types of PhACs have been intentionally designed to affect living systems at relatively low doses. As a result, PhACs at environmentally relevant concentrations ($\mu g\ L^{-1}$ to ng L^{-1}), have been recently identified as potentially toxic for aquatic organisms. In addition, bioaccumulation is possible for some specific compounds. This study aims to investigate the use of solitary ascidians, highly efficient filter-feeding sessile invertebrates, as biological indicators for PhACs. Our goal is to exploit the cosmopolitan distribution of invasive ascidians in order to develop a uniform method using the same bio-indicator species, across a wide geographic scale. Our objectives are to evaluate the spatial and seasonal variation of ascidians' ability to accumulate PhACs and to identify protein expression in response to PhACs accumulation using a proteomic approach. We developed an analytical method for detection of PhACs in ascidians' tissues using pressurized liquid extraction and analysis by high-pressure liquid chromatography. We detected three PhACs, Carbamazepine, Bezafibrate and Diclofenac, during preliminary testing. We are currently analysing collected samples in order to present our results at the SETAC Europe 29th annual meeting. Analysis of protein profiles of ascidians under laboratory controlled exposure experiments is also in progress. To our knowledge, this is the first time ascidians are used as bio-indicators for PhACs contamination. Ascidians hold a promising potential for detection of PhACs, before causing irreversible ecological damage. Since ascidians are the closest living relatives of vertebrates, there is an advantage studying their response to PhACs contamination over other marine invertebrates, giving the opportunity to better understand the possible effects on marine vertebrates and on humans.

TU055

Bioaccumulation modelling of selected metals (As, Cd, Cu, Ni, Pb, Zn) in coastal and estuarine food webs. Case studies

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The study aimed to bring together knowledge gained from the results of the bioaccumulation modelling and field campaigns in order to obtain a more complete picture of bioaccumulation phenomena in aquatic food webs in two case studies. The first one located in estuary of the Lagoon of Venice, Italy and the second one in the coastal area of Liaodong bay, China. Both represent interesting scenarios for ecological exposure modelling due to the availability of local data on metal concentrations in water, sediment and in site specific aquatic species. Also both areas are economically important and affected by long-term and large-scale industrial activities posing potential ecological as well as human risks following the exposure to a multitude of environmental contaminants. MERLIN-Expo was selected to model exposure to metals and metalloids due to its novelty and broad application domain (Ciffroy et al., 2016). The model was dynamically run using observed exposure concentrations (sediments, water), and was parameterised probabilistically with site and organism specific data. Subsequently, the simulation results were evaluated against measured bioaccumulation data in soft tissues of the selected organisms. Finally, the Morris and extended Fourier Analysis (EFAST) were used to account for variability in selected parameters of the bioaccumulation model on the simulated results. We have obtained satisfactory simulation results especially for bioaccumulation of Zn and As, which showed the best agreement with measured values in aquatic organisms. The sensitivity analysis complemented the modelling of bioaccumulation by allowing to identify crucial parameters for simulating the internal exposure of organism to metals. Food assimilation efficiencies of selected aquatic species seem to be the key components of the modelling, driving the variability of internal exposure to the selected metals and metalloids.

TU056

A 16th century shipwreck mercury legacy and present mercury bioaccumulation in intertidal species

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Over the centuries, mercury (Hg) has been used for a variety of purposes being employed in the manufacturing of thermometers, barometers, explosives, playing also a significant role in dentistry, pharmaceutical industry and agriculture. Furthermore, Hg is commonly used in gold and silver mining processes to separate the metal particles from the sediments (aquatic and terrestrial) through a process called amalgamation. In Spanish colonial times, the Hg necessary to the mining process was carried in galleons along the Atlantic, from Spain to the New World. However, some of these vessels occasionally sank due to storms or collisions with reefs, potentially releasing the stored Hg. Once in the environment, Hg has the particularity to bioaccumulate and biomagnify in aquatic food webs, this way Hg concentrations found in predator species can be millions of times higher than those observed in surface waters. Furthermore, biomagnification of Hg can lead to toxic concentrations in fish and fish-eating wildlife. It is possible to monitor Hg presence in the marine environment through the analysis of Hg concentrations in the tissues of the organisms that are able to accumulate this pollutant (accumulation bioindicators). There are several criteria for an organism to be considered an accumulation indicator: (1) sessile or at least restricted mobility, (2) ubiquitous and sufficiently abundant in the sampling area, (3) available in all seasons, (4) ease of sampling, (5) large body to provide sufficient material for chemical analysis, (6) high capacity to accumulate contaminants above environment levels, (8) predisposition to retain contaminants for a sufficient time after reduction in the environment. In the present study, the authors focused on the evaluation of Hg concentration in marine sediments and among different marine species (*Asparagopsis armata*, *Ulva lactuca*, *Littorina striata*, *Patella candei*,*Paleamon elegans* and *Pachygrapsus marmoratus*) collected in a bay where a 16th century shipwreck can be found and in other bays without the presence of shipwrecks. The Hg concentrations found in the sediment and in the marine species collected in the shipwreck area was significantly higher in comparison to those found in the other bays. The shipwreck Hg legacy seems to have an influence on the present Hg distribution in the sediments and in the intertidal species.

TU057

Temporal changes in dissolved elemental mercury at a coastal aquaculture site in the Grado Lagoon, northern Italy

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Mercury can be lost from ecosystems through the process of photoreduction to its volatile elemental form (Hg(0)) in natural waters which is primarily controlled by abiotic reactions with ultraviolet (UV) radiation. There is little mercury photoreduction research available for contaminated coastal marine systems. Grado lagoon, northern Italy, is an aquaculture area with high total mercury concentrations in sediment due to past cinnabar mining activities at Idrija (western Slovenia). Two sites (one highly Hg contaminated, VN1, and one with lower contamination, VN3) located within a lagoon fishfarm were analyzed for temporal changes in dissolved elemental mercury ($Hg(0)_{aq}$) and its relationship with UV radiation in May of 2018. Solar radiation spectra were continuously quantified using an OceanOptics USB 4000 spectroradiometer and aqueous Hg(0) concentrations ($Hg(0)_{aq}$) using a Lumex 915-RA with headspace analysis and an equilibrium partitioning model with temperature-corrected Henry's law constant. It was found that the small pH changes observed were significantly correlated with $Hg(0)_{aq}$ concentrations at both sites however this was likely due to auto correlations with the daily cycle of microbial respiration and carboxylic acid concentrations. It was found that $Hg(0)_{aq}$ concentrations ranged between 237.6 - 401.5 pg L^{-1} (mean = 331.4 pg L^{-1}; SD=42.94; n=13) at the more contaminated site, VN1, and showed a diurnal trend. Total dissolved mercury at this site ranged between 3.85 - 28.22 ng L^{-1} (mean=7.7; SD=6.17 ng L^{-1}; n=13), which resulted in a % Hg(0) ranging between 0.84 – 8.24 %. At the lower mercury site, VN3, $Hg(0)_{aq}$ concentrations ranged between 200.3 – 321.52 pg L^{-1} (mean=279.49 pg L^{-1}; SD=46.99; n=14). Total dissolved mercury at this site ranged between 2.86 – 9.13 ng L^{-1} (mean=5.04; SD=2.06 ng L^{-1}; n=14), which resulted in a % Hg(0) ranging between 2.89 – 8.91 %. In both cases the $Hg(0)_{aq}$ concentrations in water were higher in the central part of the day and positively correlated with incoming UVB and UVA radiation measurements (Site VN1 Pearson r = 0.48, p=0.09, n=13; Site VN3 Pearson r=0.70, p=0.01 n=13). In conclusion, it was found that % Hg(0) relative to total mercury concentrations were similar at the contaminated coastal aquaculture site to other contaminated areas in previous work, but were substantially higher than those observed at other uncontaminated sites.

TU058

Salinity influences on mercury photochemistry in estuarine rivers in Minas Basin, Canada

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Mercury (Hg) loss from natural waters can occur through photoreduction that is controlled primarily by ultraviolet (UV) radiation. The retention of Hg in aquatic systems is largely affected by its reduction-oxidation photochemistry, and the chemical characteristics of estuarine water may significantly affect the photochemistry of Hg and its eventual accumulation in food webs as methylmercury, a potent neurotoxin. Currently, there are limited data on Hg photochemical cycling in estuarine ecosystems compared to freshwater ecosystems. To address these knowledge gaps, the photochemical kinetics of mercury speciation with respect to salinity and dissolved organic matter (DOM) were quantified. It was hypothesized that the rate and absolute amount of photochemical Hg reduction in estuarine waters is facilitated by DOM and inhibited by salinity. Water samples from the Cornwallis tidal river estuary in the Minas Basin, Nova Scotia, Canada were taken in July of 2018. Using tangential ultrafiltration, an array of salinity and DOM conditions were attained and then irradiated over an environmentally-relevant range of five UV intensities between 300–400 nm. Gaseous elemental mercury (Hg(0)) was analyzed every five minutes over a period of 24 hours using a Tekran 2573B CVAFS automated mercury analyzer. Gross photoreduction and photooxidation reversible pseudo-first order reaction rate constants and total photoreducible Hg in each treatment were determined using curve-fitting software. These data and the correlations with DOM and salinity change and will be presented. The proposed research will provide fundamental data and rigorous experimental testing which can then be used to predict Hg retention and accumulation in estuaries.

TU059

Mitigating measures to eliminate organochlorine pesticides in surface water of developing countries in Southeast Asia

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Importance of pesticides are well recognized, as these were developed for very reasonable purpose. But unknown to some farmers, there are also quite a handful of banned organic pesticides, as these give adverse effect to human and the environment. This study aims to assess and analyse the residual banned organochlorine pesticides unintentionally disposed or transported through surface runoff from farms in the upstream to receiving waters. In the pesticides

assessment, passive samplers were used instead of the traditional grab sampling. The sampling were conducted uninterrupted for 34 days in Cagayan de Oro River, Philippines and Song Can Tho, Vietnam. HPLC, GC-MS MS AND LC-MS MS were used to quantify the pesticide concentrations. The time weight average of the organochlorine restricted pesticides were measured in trace amounts. What is alarming is that these banned organic pesticides were already banned for almost two decades. These were prohibited because these organochlorine compounds persist, bioaccummulate and have adverse effect to human and the environment. It is recommended that responsible government agencies should invest in awareness raising activities on good practices and management of pesticides use to farmers, regular monitoring of pesticides and strict implementation of existing environmental laws and policies.

TU060

Multi criteria assessment to optimize plant protection strategies: A case study with apple orchards

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Monitoring programmes have shown that pesticide concentrations in surface water have exceeded water quality criteria. Pesticide emission to surface water can be decreased by reducing pesticide use and/or by reducing emission, e.g. via specific risk mitigation measures. Reducing the use of pesticides can be very challenging for the production of crops. E.g. for apples, even in organic production pesticides are applied 20-30 times per year to protect crops against diseases and pests. However, risk mitigation measures alone might not be sufficient to achieve good water quality in small streams in agricultural catchments. Therefore, optimized plant protection strategies are being developed with reduced pesticide use. To quantify the contribution of optimized plant protection strategies on risk reduction and to understand potential economic and environmental trade-offs related to these strategies, a multi criteria assessment method (MCAM) was conducted: ecotoxicological risks as well as environmental and economic impacts were assessed. The MCAM was applied to apple orchards in Switzerland. We compared five different plant protection strategies (low-input, high-input, average organic, optimized organic and risk-optimized chemical scenario) with an average chemical plant protection strategy (reference scenario). For each scenario, ecotoxicological risks in surface water, soil and off-crop habitat were assessed with the model SYNOPS. Environmental impacts were assessed with life cycle assessment (LCA), focusing on ecotoxicity, terrestrial in-crop biodiversity, use of minerals and energy, greenhouse gas potential and eutrophication. For the economic assessment, profitability, resource intensity (capital and workload) and variability (fluctuations in yields and revenue) were considered. Finally, the results from these very diverse quantitative methods were translated into a comparative assessment, where each crop protection strategy was rated compared to the reference scenario. This allowed to assess and compare crop protection strategies from different angles. In this study we show the important role of choice of plant protection strategy for the risk to aquatic and terrestrial environments. Combined with appropriate risk mitigation measures, improvements in water quality can be achieved. The MCAM also supports decision-making by early identification of potential trade-offs with other environmental impacts or economic performance.

TU061

Marine debris in Central Chile: Characterization of macroplastics in Concepción Bay

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Plastic debris can be transported over long distances from the sites of origin and it accumulates through the ocean currents in oceanic turns. Recent studies have been devoted to characterize the Anthropogenic Marine Debris (AMD) along the Chilean coast, where it has been shown that the most abundant AMD are plastics and cigarette butts which can be attributed to local sources with increase over time. In this study we have characterized plastic debris (PD), in five beaches of Concepción bay in central Chile during two different periods (spring 2017 and summer 2018). Samples of PD were collected and analyzed physically by abundance, shape and colour. The sampling was carried out using three transects and three quadrants (3x3 m) for each beach. The physical analysis was carried out with a high resolution optical microscope with an integrated camera, Leica DM 750 and the chemical analysis was conducted with Agilent Cary 630 Fourier transformed infra-red spectrometer (FT-IR). The preliminary results showed in general, the highest abundance of PD was detected in summer with average values of 3 ± 1.3 items/m^2 and 1 ± 1.4 items/m^2 in spring. The physical characterization showed the most frequent size was PD < 2.5 cm (46%) (mesoplastic), with the most frequent shape for plastic pieces (48%) and others (46%) like pieces of polystyrene and elastic. In addition the predominant PD colour found was white (22%) and others (20%) like purple and orange. The chemical analysis of PD showed that the most frequent plastics identified were mostly polipropilene (38%) and polyethylene (19%). These data are the first information of PD in touristic beaches of the Concepción bay and are also comparable with other studies reported in the scientific literature.

TU062

Mixture of metals in coastal water of Kaldvellfjord, Norway - speciation, bioavailability and uptake in organisms

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Metal rich runoff as a result of deposited iron sulphide-containing bedrock after construction E18 highway in southern Norway entering coastal water in Kaldvellfjord. Thus, aquatic organisms in the costal water were exposed to a number of trace metals. The focus of this study was to identify the interaction of trace elements in mixtures on speciation, bioavailability and on uptake in aquatic organisms in the coastal waters as a function of salinity to improve the risk assessment of Kaldvellfjord. Investigation in Kaldvellfjord demonstrated good correlation between salinity in the surface water and water flow in the river, decreasing during flooding events. Controlled experiment based on mixing runoff water with seawater demonstrated dilution of trace metals, however specific changes in speciation and distribution occurred at increased salinity. Comparison of results with mixing of artificial runoff water containing single metal and mixture of metals demonstrated that the form of metal is different when present alone compared to the mixtures. Thus, metals behave competitively and this affects speciation and the bioavailability. Exposure of fish to the estuarine water demonstrated uptake of several metals. Cumulative risk assessment study, identified aluminum as a main driver in range of several mg/L, however iron (Fe), copper (Cu), nickel (Ni), zinc (Zn) and manganese (Mn) present in the range from 100 to 1500 μg/L were assessed also to have a significant impact on fish and invertebrates. In addition, several rare earth elements from the runoff water was accumulated in fish and assumed to contribute to the cumulative risk.

TU063

Comparison of ASV and ISE methods for predicting Cu bioavailability in seawater

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The bioavailability of dissolved metals is determined by their chemical speciation, in particular on the concentration of free metal ion according to bioavailability models such as the free ion activity model (FIAM) or the biotic ligand model (BLM). In seawater, at constant salinity and pH, the inorganic complexation of divalent metals such as Cu remains constant, and the main factor causing variations in metal speciation is the presence of organic ligands, such as dissolved organic matter (DOM). The two most widely used techniques in Cu bioavailability studies are anodic stripping votammetry (ASV) and potenciometry using Cu^{2+} ion selective electrode (ISE). However, these two techniques do not have the same detection window and have frequently resulted in contrasting results with differences of even several orders of magnitude. This has lead to uncertitude about which technique is giving correct resuls, so that it can be relied on to predict Cu bioavailability in seawater. This presentation will offer a thorough review of the literature about the studies about Cu bioavailability in seawater that have used ASV and/or ISE for the measurement of free or labile Cu, with the aim of compiling evidence about which of the methods works better for the prediction of Cu bioavailability. In addition, some new experimental work will be presented that shows that the results of both methods can be reconciled.

TU064

Possible sources and trophic dynamics of multiple trace metals in coastal food webs from Patagonia and Antarctica

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Antarctic coastal biota is diverse, composed mainly of species of molluscs, polychaetes, amphipods, bryozoans, sponges, isopods, crustaceans, ascidians, pycnogonids, fishes, marine mammals and seabirds. On the other hand, food webs in the Patagonian coastal region are even more complex than in the Antarctic, both regions considered pristine ecosystems. There is a lack of information for trace metals fate and effects on those regions of the southern hemisphere. Even more trace metal biomagnification processes in marine food webs in remote/pristine zones in the southeaster Pacific coast, including near Patagonia and in Antarctica are even more scarce. The differences in the composition of the food web structure between Antarctica and Patagonia, along with physical processes and the input of freshwaters to coastal systems might influence trophodynamics of Hg, Cd, Ta, Pb. Most of the scientific research have shown that Hg biomagnifies through food webs and has a positive correlation with higher latitudes. Despite that fact, we showed that freshwater inputs in Patagonia and Antarctica have certain influence on this process, with higher TMS (0.243 and 0.217 respectively) near freshwater outlets (analysing trace metal concentrations vs $d^{15}N$). At the same time other trace metals have been assumed to present similar trophic processes than Hg, but despite evidence shows Cd biomagnifies (TMF, 0.66-2.85), it does only within the macroinvertebrate group in these food webs. When we did include fish in the analysis, we observed a significant dilution of Cd on the higher trophic organisms (TMF −0.81 ± 0.14). Bioaccumulated lead in Patagonian and Antarctica food web have a clear anthropogenic source, since $^{208}Pb/^{207}Pb$ versus $^{206}Pb/^{207}Pb$ signatures differ greatly from the geological background found in the same areas. Even more, trace metals used by industry and exploited far away from the studied sites have been found. Tantalum (Ta) is a Technology-Critical Element (TCE), which we know practically nothing on its fate and possible effects in wildlife. We have shown that there is a trophic transfer of this element with TMS ranging from 0.004to 0.018. All this information has important implications for regulatory purposes, as Patagonia and Antarctica are not regions with industrial development. At the same time these regions are crucial to understand biogeological processes in pristine areas and to assess their possible impacts in wildlife.

TU065

Toxicokinetics and bioacummulation of cadmium and mercury in marine microcrustaceans Artemia sp. and Mysidopsis juniae

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The species used in this study, the marine microcrustaceans *Artemia* sp. and *Mysidopsis juniae* are fairly utilized in Ecotoxicology and they are characterized by low and high sensitivity, respectively, to various contaminant groups. *Artemia* sp. nauplii stage II (48 h old) and juvenile mysids *Mysidopsis juniae* (0-7 days old) were exposed to cadmium (Cd) and mercury (Hg) in water at 50 ɂg Cd.L^{-1} and 5 Ղg Hg.L^{-1} (the mysids No observed effect concentrations (NOEC) for both metals); besides this, mysids were simultaneously exposed to previously contaminated food (Artemia). Organisms were sampled at different time points to determine their internal metal concentration over time. The experiments for *Artemia* sp. and mysids lasted 48 h and 96 h, respectively. The first half time of each species exposure, 24 h for *Artemia* and 48 h for mysids, was considered the uptake phase. At the end of this phase, crustaceans were transported to uncontaminated seawater for the elimination phase outset. An one-compartment model was adopted to describe Cd and Hg kinetics. Bioconcentration factor in *Artemia* sp. nauplii was superior for Cd than Hg. In both species, Cd bioconcentrations decreased at the elimination phase On this phase, Hg concentrations remained stable in *Artemia* sp. and *Mysidopsis juniae*. Accordingly to the bioaccumulation observed, Cd is detoxifiable for both species and Hg was more transferred from Artemia to *Mysidopsis juniae* via food and water than Cd. The results obtained in this study evidenced the high capacity of Hg transference throughout the food chain and its persistence in crustaceans.

TU066

Integrated sediment risk assessment by correlating metal bioaccumulation to DGT passive sampling and varying sediment properties

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The management and remediation of contaminated sediments is a rather complex subject in which integrated assessment methods have been experienced to be crucial for ensuring a decent balance between reasonable costs and environmental benefits and safety. In this regard, the importance of the actual bioavailability of sediment bound contaminants became more and more apparent during the last decades as highly contaminated sites can be characterized with a low toxic potential due to low ratios of weakly bound or free contaminant fractions. For metals, the analysis of parameters such as pore water concentrations, acid extractable metals (AEM), acid volatile sulphides (AVS) and organic carbon have been experienced to be useful predictors, however with varying effectiveness (especially in more oxidized surface sediments) between the different metals. A promising alternative has been found in passive sampling devices, including Diffusive Gradient in Thin film samplers (DGTs). DGT passive samplers give an indication of metal flux behaviour between the different compartments over time and the concentration of bioavailable metal fractions in the sediment by binding divalent metals, weakly-bound to the sediment or present in the pore water, on a chelex ion-exchange resin. In February and March 2019, a laboratory experiment will be conducted in which the toxic potential of a set of about 20 different natural sediments with known biochemical properties (fresh and brackish) will be tested. This will be done by taking the different indicative parameters into account, including pore water concentrations, AVS, AEM and total metal concentrations. The different parameters will be compared to metal concentrations measured by DGT passive samplers. Furthermore, two mussel species (*Corbicula fluminea* and *Dreissena bugensis*) and two sediment digesting worm species (*Hediste diversicolor* and *Lumbriclulus variegatus*) will be exposed to the sediments over 28d. The former parameters will be compared and correlated to the total metal body burden and mortality of the exposed organisms. The results of this study are expected to increase the insights in metal release behaviour over time, the accumulation of such by benthic organisms, and the ability of DGT passive samplers to predict bioaccumulation concentrations. The study aims at contributing to the improvement of water system management and remediation activities for both freshwater and brackish aquatic systems.

TU067

Biomarkers as indicators of the State of health of Pteria sterna in Ensenada de la Paz, B.C.S. Mexico

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The urbanization process of Ensenada de La Paz has increased the contribution of xenobiotics in the coastal zone, causing an increase in the levels of xenobiotics that together with the climatic changes, constitute a risk for the health status of the pearl oyster *Pteria sterna*, resource of great economic importance in this area. In this work an evaluation of the effects of the contaminants present in the cultivation sites, in the health status of *P. sterna* was made by means of the evaluation of biomarkers. Organisms were collected in two locations of Ensenada de la Paz, a site in front of Isla Gaviota (24º17'21.75"N and 110º20'26.9"W) and the other site in the Marina of La Paz (24º09'09.77 " N and 110º19'25.75 "W). 20 organisms were collected from each site during 2 seasons (summer and winter). The condition index was evaluated. The degree of lipoperoxidation (TBARS), the activity of antioxidant enzymes: SOD, CAT, GPx, AChE activity and MN frequency were also determined. The results obtained in the condition index, lipoperoxidation, CAT, AChE and MN in the two sites showed a significant difference ($p < 0.05$). The pearl oysters with the highest growth rate were those of Isla gaviota. A higher degree of lipoperoxidation was observed in the gills of molluscs collected in the Marina. The activity of CAT was greater in these mollusks. AChE activity was lower in pearl oysters of the Marina. The frequency of MN was higher in the pearl oysters of the Marina. The data obtained in the evaluation of lipoperoxidation, AChE, SOD, CAT and MN in *P. sterna* of La Marina could be related to the presence of metals that cause negative effects on their health status.

TU068

Impact of strongly elevated CO2 concentrations on a invertebrate community tested in marine mesocosms

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In an effort to reduce harmful emissions and increase energy efficiency, the application of Liquefied Natural Gas (LNG) as fuel for ship engines is being studied in combination with an underwater exhaust system to reduce the ship's water resistance. The exhaust gas from LNG powered ships consists for a substantial part of CO_2, which may significantly increase CO_2 levels in the water around the ship. Most studies on elevated CO_2 concentrations use only moderate CO_2 exposure levels to mimic estimated future global conditions, around 936 ppm in 2100, caused 0.5 units decrease in global average ocean pH. Although the potential impact of underwater exhausted CO_2 is not known yet, it might locally result in higher CO_2 concentrations than usually studied. To gain more insight in the local impact of higher CO_2 concentrations, a benthic mesocosm study was performed under a pH range of 8.6 to 5.8. Community compositions changed with increasing CO_2 concentrations. Benthic polychaetes formed the least sensitive group and were able to take advantage of the reduced competition and became the dominant group at pH levels 7.1 and 6.6. Their larvae dominated the zooplankton community. At the next higher exposure level even the polychaete population collapsed, and a phytoplankton bloom developed probably facilitated by to the lack of grazing and the flux of nutrients from decaying organic material. The periphyton community was not able to take advantage under these conditions. Gastropods that comsuming periphyton suffered directly from higher CO_2 levels as indicated by shell damage, however their growth was not directly related with pH, but was positively correlated with the availability of periphyton. Our study was conducted in relatively small systems without water replacement or exchange, and therefore represents a worst case situation as can occur in an isolated ecosystem. In this situation a clear impact was observed when CO_2 injection resulted in pH levels of 7.1 or lower for 49 days. The impact of underwater release exhaust gasses on the water quality depends on various factors including the local biological and abiotic conditions. This study focused on impact of CO_2 only and future studies will be conducted to gain insight in the impact of other components of actual exhaust gasses. The final goal is to assess the boundaries, where the advantages of underwater exhaust gasses as ship hull lubricant can be applied without negative impact on the marine ecosystem.

TU069
DANUBIUS-RI: Making River-Sea Systems Work
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River-sea systems (RSS) comprise river catchments, estuaries/deltas, lagoons and coastal seas and provide a vast array of valuable services ranging from food supply and flood protection to aesthetic value. Human uses can have several negative impacts on RSS including eutrophication, pollution by microcontaminants, altered river morphology and hydrology, and overall loss of ecosystem services. Our ability to understand and manage such impacts has been constrained by disciplinary boundaries and a lack of interdisciplinary initiatives to undertake, develop and apply the necessary fundamental and integrated research. The International Centre for Advanced Studies on River-Sea Systems (DANUBIUS-RI) will be a pan-European distributed research infrastructure dedicated to supporting interdisciplinary studies of RSS. An associated European Coordination and Support Action project is currently being funded to develop a coherent, effective and relevant scientific agenda for RSS. The DPSIR framework (Drivers, Pressures, State Changes, Impact and Human Response) has been used to identify overarching challenges that the research community will have to address to ensure that RSS remain functional over a long period. Climate change and extreme events along with activities linked to basic human needs and societal functioning (e.g., fisheries, transport, agriculture, power generation, tourism, urbanisation) were identified as key drivers in determining the various pressures affecting RSS and the associated state changes, impacts and possible human responses. Based on the complex network of relationships and feedbacks identified by the DPSIR approach, four overarching challenges in making (or maintaining) RSS functions have been identified: climate change, water sufficiency, sediments and their management, and ecosystem health. This scientifically and societally relevant general framework will allow definition of research questions, needs and practical aspects for preserving RSS and the services they provide.

Fish Model Species in Human and Environmental Toxicology (P)

TU070
The fish embryo toxicity test under flow-through conditions - development of a biowellplate for whole-organism bioassays
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Whole-organism bioassays have become important methods in compliance testing of industrial chemicals and plant protection products, but also in the assessment of environmental quality. With few exceptions, these systems are discontinuous. They require exposure in small units, such as multiwell plates, for prolonged incubation periods. Flow-through conditions with a constant renewal of the exposure medium improve the stability of compound concentrations and can also improve the applicability of whole-organism bioassays in large-scale study designs. This study introduces a biowellplate (BWP) designed for the incubation of organisms under flow-through conditions in a multiwell plate format. The BWP was utilized to perform prolonged fish embryo toxicity tests (FET) with zebrafish (*Danio rerio*) embryos. The BWP was CNC-milled from PMMA plates. The design includes 8 single wells with dimensions comparable to standard wells of a 96 well plate. At the bottom side, each well is connected via two bypass channels to an individual inlet port that provides a continuous flow of fresh exposure medium. The wells are enclosed by a temperature channel that provides continuous circulation of warm water through the plate for optimal temperature conditions.The BWP bottom side is sealed to the middle side and the top side is manually closed by a silicon polymer sheet. The BWP was assembled in a customized housing case that can hold all parts necessary for the operation of the BWP and evaluation of the testorganisms. Preliminary tests found the BWP to be tigth and suitable for the utilization of zebrafish embryos for prolonged incubation periods. In a first proof of concept study the functionality of the system under permanent operation will be tested. Initial pretests will test for the optimal flow-rate, which was defined as the maximum medium exchange rate at which zebrafish embryos develop normally until 119 hours post fertilization. After optimization of the setup and configurations, prolonged fish embryo toxicity tests with zebrafish embryos and a set of well described chemicals will be conducted. This will allow for an evaluation of the BWP's performance in comparison to standardized protocols for whole organism bioassays. In this study, we present a novel layout and setup for a multiwellplate that allows for the prolonged incubation of fish embryos under flow-through conditions that also provides wells large enough to conduct behavioral assays with hatched embryos.

TU071
Three- and four-ring PAHs differentially modify ventricular action potentials and ion currents of rainbow trout (Oncorhynchus mykiss) heart
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Polycyclic aromatic hydrocarbons (PAHs) are contaminants that occur frequently in aquatic environments. They have been shown to affect the development and function of the heart in fish. We studied the effects of two three-ring PAHs, phenanthrene and retene, and two four-ring PAHs, pyrene and fluoranthene, on the electrical characteristics of rainbow trout (*Oncorhynchus mykiss*) ventricular cardiomyocytes. Effects on cardiac action potential (AP), and sarcolemmal sodium (I_{Na}), calcium (I_{Ca}) and potassium (I_{Kr} and I_{K1}) currents were studied by whole-cell patch-clamp method. The effects on AP and ion currents varied between compounds, and our results suggest there may be differences between species as well, as in rainbow trout phenanthrene had no effect on AP duration. All studied compounds inhibited I_{Kr}, and they therefore may have the potential of causing bradycardia and arrhythmias in rainbow trout heart. This means that in addition to petrogenic mixtures, also pyrogenic mixtures with high concentrations of pyrene and fluoranthene may disrupt cardiac function in fish. Retene is an AhR agonist, and besides this transcriptomic mechanism of toxicity, it may be cardiotoxic by a direct effect on cardiac ion channels.

TU072
Tissue-specific gene expression of CYP1A1, 3A27 and 3A45 in juvenile rainbow trout
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Anthropogenic activity results in continuous exposure of aquatic organisms to low doses of biologically active compounds in natural ecosystems. The Cytochrome P450s (CYP) are an important family of enzymes, with the main function to catalyse reactions involved in oxidative metabolism of endogenous and exogenous compounds. Some of the piscine CYP genes are well characterized, however little is known with respect to their importance. In the present study we examined the expression of three CYP genes, CYP1A, CYP3A27 and CYP3A45 in six rainbow trout tissues: hepatic, gill, intestine, brain, heart and gonad. The mRNA was expressed in all investigated tissues. The CYP1A1 mRNA expression was highest in the liver and gonads. Interestingly, tissue-related expression pattern differed between CYP3A27 and CYP3A45, with CYP3A27 highly expressed in the liver, heart and gonads compared to CYP3A45. For CYP3A45, brain tissue had the highest expression of CYP3A45, among all selected genes. These differences might be related to the physiological function of the specific CYP isoforms in the tissue. Further studies are needed to determine the significance of the observed tissue-specific expression pattern of the investigated CYPs, and how this relates to detoxification of environmental pollutants. ***Keywords:*** cytochromes, detoxification, gene expression, *Onchorincus mykiss* Acknowledgement - The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA "(No. CZ.1.05/2.1.00/01.0024), "CENAKVA Center Development "(No. CZ.1.05/2.1.00/19.0380), "CENAKVA II "(No. LO1205 under the NPU I program), and "Development of USB - International mobility (No. CZ.02.2.69/0.0/0.0/16_027/0008364).

TU073
Trans-omics investigations to predict adverse outcomes of 17α-ethinylestradiol in early life stage fathead minnow (Pimephales promelas)
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The growing number of emerging chemical contaminants is becoming an increasing concern to water managers due to their potential toxicity to non-target organisms, pseudo-persistence and discharge into surface water systems. The main issue with current approaches to testing the risks these chemicals may pose to human or wildlife is that they rely on live animal testing that is expensive, time consuming and of ethical concern. A promising concept to address this issue is the investigation of the mode of action of a chemical to predict an apical outcome of regulatory relevance; however, there is a need to calibrate and validate this approach by conclusively linking this mechanistic information to toxic outcomes. This study applied a trans-omics approach that aimed to link changes at the gene expression level through multiple organizational levels to physiological and apical changes using 17α-ethinylestradiol (EE2) as the model compound and fathead minnow (FHM) as the model organism. Specifically, FHMs were exposed to 4, 20 and 100 ng/L EE2 from freshly fertilized eggs to the early juvenile stage. Subsamples of FHM embryos were collected 4-days post hatch (dph) for transcriptomic, proteomic and metabolomic analyses, and the rest of the embryos were continuously exposed to EE2 until 28 dph for histological and apical outcome examination. Genes and proteins associated with coagulation, a variety of protein processing and metabolic processes, and necrosis were significantly dysregulated. Gene set enrichment and pathway analyses (GSEA) at the transcriptome and proteome levels showed a number of common overrepresentations corresponding to observed apical outcomes. For instance, EE2 significantly induced vitellogenin (VTG) gene expression, with a complementary induction of VTG isoforms at the proteome level. Histological observations of the liver showed damages that may be linked to overrepresented gene ontologies and pathways such as increased hepatocyte basophilia and the abundance of eosinophilic proteinaceous fluid within the sinusoids which are known responses to estrogen-induced upregulation of VTG, as well as the absence of glycogen-type vacuolation due to increased energy demand. Results suggest trans-omics analyses is a powerful tool in furthering our understanding and predicting phenotypic responses of contaminants. The results of this study contribute towards the realization of the EcoToxChip: a toxicogenomics tool for chemical prioritization and environmental management (www.ecotoxchip.ca).

TU074
Aryl Hydrocarbon Receptor-Mediated Effects of Dioxins on Liver in Zebrafish Embryos
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Exposure to dioxins and dioxin-like compounds, a series of widespread environmental contaminants, could cause adverse health effects, including hormonal problems, infertility and cancer. Increasing evidence suggests that dioxins induce liver damage mediated by aryl hydrocarbon receptor (AhR) signalling. However, limited in vivo and in vitro platforms (with the advantage of time-efficient and cost saving) exist to assess the liver damage by dioxins. Here, we used zebrafish embryos to demonstrate adverse impacts of dioxins on the liver, and explore the relationship between liver damage and AhR signalling. Zebrafish embryos were exposed to the hepatotoxic reference compound-Thioacetamide (TAA) and to three typical dioxin and dioxins-like compounds (Benzo[a]pyrene(BaP); 2,3,7,8-Tetrachlorodibenzo-para-dioxin and PCB126). Histopathology revealed compound and life-stage-specific effects indicative of toxic injury in the livers of zebrafish larvae. Quantitative PCR was used to compare gene expressions related to AhR and liver damage. Using histology analysis, it was observed that low concentrations of BaP could induce liver damage in zebrafish embryos. CYP1A (involved in AhR activation) gene expression was significantly up-regulated in response to the three dioxin treatments; whereas with TAA treatment eliciting acta2 (involved in liver fibrosis) gene expression decreased. The zebrafish embryo model combined with histology and gene markers could be an alternative method to assess the toxicity of dioxins.

TU075
Concerns for Di-isononyl phthalate contaminated diet in marine fish: focus on the effects on both the hepatic lipid metabolism and the muscle proteolysis of male gilthead sea bream (Spaurus aurata)
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Many chemical compounds used in the production of plastics and resins have the ability to interact with the endocrine system, acting as Endocrine Disrupting Chemicals (EDCs) and thus representing a high-priority research area. In the present study, we analysed the effects of a dietary administration of a common plastic additive tDi-isononyl phthalate (DiNP) on fish physiology metabolism. DiNP was administered for 21 days via the diet (4 and 4000 μg DiNP/kg body weight/day) to adult male gilthead sea bream (*Sparus aurata*), to assess its effects on the hepatic lipid metabolism and muscle proteolysis. Our findings showed the ability of DiNP to alter the hepatic structure and its biochemical composition, as well as modulating the expression level of genes involved in lipid biosynthesis and endocannabinoid metabolism. In addition, since gilthead sea bream represents one of the most valuable species by consumers, given its fillet quality and nutritional properties, changes induced by DiNP exposure on muscle macromolecular structure and its ability to disrupt the proteolytic system were investigated. Results showed that DiNP could substantially change muscle composition and affect fillet tenderization. These results provide a macromolecular fingerprint of the liver and the muscle of fish fed with DiNP and highlight the negative effects of contaminated diet on physiology and metabolism, contributing to assess the potential ecological impact of emerging pollutants in the aquatic ecosystems and their effects on marine food resources. In addition, the results may suggest a set of biomarkers as possible innovative endpoints for the development of novel OEDCS test guidelines.

TU076
Tissue Metal Concentrations and Anti-oxidant Enzyme Activity in Atlantic Sharpnose Shark
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Metals occur naturally in the environment; however, anthropogenic practices have resulted in increased metal concentrations in sediments, waterways, and biota. Sharks are important species, both recreationally and commercially. Quantifying tissue metal concentrations in these organisms may reflect metal transfer in aquatic food webs. Extensive research has been conducted on mercury toxicity in sharks; however, reference levels of other metals have not been established in many species. In this study, concentrations of nickel, copper, lead, cadmium, selenium, silver, zinc, and aluminum were measured in the muscle tissue of *Rhizoprionodon terraenovae* (Atlantic sharpnose shark), collected from Virginia to Texas. A total of 165 samples were analyzed for correlations among metals, size/age, gender, and location of capture. Muscle samples were also analyzed for anti-oxidant enzyme activity, and results were correlated with metal concentrations. With the exception of mercury, there are no published data on tissue metal concentrations within this species along the eastern United States. This study provides reference levels of metal contaminants in the muscle tissue of Atlantic sharpnose sharks and provides insight into metal accumulation in this higher-level carnivore.

TU077
Action of two commercial insecticides phenylpyrazole (fipronil and ethiprole) on the levels of lipid peroxidation in livers of Oreochromis niloticus
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Consumers have never been so concerned about the environment. This social pressure coupled with the efforts of the scientific community to bring to light the deleterious effects of the old and massively used pesticides can cause human health and the environment has motivated the agrochemical industry to manufacture products that are less harmful without losing effectiveness. This is the case of the insecticide ethiprole, produced with the intention of being less toxic than its analogue fipronil. Reactive oxygen species (ROS) are produced from oxidation, a fundamental part of cellular metabolism. When in excess, ROS have harmful effects, such as lipid peroxidation - a process that can cause damage to several cell types. Malondialdehyde (MDA) is one of the most important intermediates during lipid peroxidation, through which it is possible to stipulate levels of lipid peroxidation in an organism. For this reason, this study had as objective to compare the action of these compounds in the levels of lipid peroxidation in liver of fish of the species *Oreochromis niloticus*. For this, a reassessment bioassay was performed, consisting of a control group, with fish exposure to clean water, and different concentrations of two commercial products - the first containing 20% ethiprole (0.1, 0.05 and 0.025 µl / l) and the second containing 80% fipronil (0.1, 0.05 and 0.025 mg / l). The results showed a small increase in MDA levels in all treatments when compared to the control, being more expressive in the highest concentrations of both commercial products; however, this increase was not statistically significant. According to the results presented here, it is evident that the tested concentrations of both commercial products studied did not significantly affect the levels of lipid peroxidation of the studied specimens.

TU078
In vivo measurement of 7-methoxycoumarin-O-demethylase activity in zebrafish (Danio rerio) embryos
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Early life stages of zebrafish (*Danio rerio)* are frequently used as alternative

model organism in (eco)toxicological and pharmacological research. Biotransformation turned out to play a critical role for the assessment of teratogenic or toxic potencies of xenobiotics. However, little is known about biotransformation capacities in early life stages of zebrafish. Since quantitative and qualitative differences in biotransformation capacities between distinct developmental stages might lead to an over- or underestimation of toxic or teratogenic potentials of chemicals, a thorough characterization of the intrinsic biotransformation capacity is imperative for a correct interpretation of toxicological data. While spatial and temporal expression patterns of xenobiotic metabolizing enzymes (e.g. cytochrome P450-dependent monooxygenases, CYPs) have previously been investigated at the genome and RNA level, only little is known about protein function or catalytic activities. Consequently, this study was designed to systematically investigate functional CYP-dependent biotransformation activities throughout early zebrafish development. To determine the constitutive spatio-temporal activity pattern and inducibility of CYP-dependent activity, zebrafish embryos (5.5 - 120 hours post-fertilization, hpf) were assessed for *in vivo* 7-methoxycoumarin-*O*-demethylase (MCOD) activity, a CYP2-dependent process in mammals. Results demonstrate that zebrafish embryos display constitutive and inducible CYP2-dependent biotransformation activity from 5.5 hpf onwards. MCOD activity appeared in a dynamic spatio-temporal pattern with activities localized in several tissues and organs (e.g. cardiovascular system, urinary tract, digestive system). In addition, pulse exposure to β-naphthoflavone (aryl hydrocarbon receptor agonist) resulted in a significant increase in MCOD activity. Results clearly indicate biotransformation capacities in zebrafish from very early stages of embryological development. This study was funded by the Ministry for Science, Research and Arts (MWK) Baden - Württemberg.

TU079

Assessing the Photoactivity of Polycyclic Aromatic Hydrocarbons (PAHs) in the Presence of TiO2 Nanoparticles

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Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent environmental contaminants with diverse physicochemistry and toxicity. In contaminated aquatic environments, PAHs frequently accumulate in sediments or are sorbed to particulates in the aqueous phase. Certain PAHs are photoactive and have photo-induced toxicity, but little is known about interactions between PAH photoactivity, sorption, environmental fate, and toxicology. Engineered nanoparticles (NPs) can behave as particle agglomerates that participate in sorption/desorption reactions in the aqueous phase, and some NPs (e.g., TiO_2-NPs) also have photoactivity. Aqueous-phase interactions between PAHs and TiO_2-NPs are of interest because they are becoming more environmentally relevant (i.e., as NPs are increasingly released into the environment), and because investigations of sorption/desorption processes, in the context of photoactivation, can provide important new information on physicochemistry of both PAHs and NPs. Our objective is to investigate PAH/TiO_2-NP interactions under UVA irradiation and determine formation of PAH photoactivated by-products (e.g., oxidized polycyclic aromatic hydrocarbons (OPAHs) and their bioactivity. Various combinations of PAH/TiO_2-NP preparations were exposed to UVA, and changes in gene expression of genes involved in Phase I metabolism (cytochrome P450 *cyp1a1* etc.) and Phase II metabolism (epoxide hydrolases *ephx1* and *ephx2* etc.) in early life stages of zebrafish were assessed over time. These data have shown significant induction of *cyp1a*, *ephx1*, *ephx2*, and *sod1* genes in larvae exposed to irradiated PAH/TiO_2-NP solutions, specifically anthracene, benzo[a]pyrene, and fluoranthene, compared to irradiated PAH solutions. Gene expression among the five different PAHs exhibited PAH specific patterns of induction, with phenanthrene and pyrene having little to no amount of gene induction of any of the genes assessed, even in the presence of TiO_2-NPs. Additionally, gas chromatography-mass spectrometry (GC-MS) will be used to identify and quantify photoactivated PAH by-products at specific time points to further support these gene expression data. The exploitation of biological responses and analytical chemistry to investigate changes in PAH physicochemistry and PAH/TiO_2-NP interactions processes will provide novel insight into these processes tested directly within the environmentally relevant concentrations and aqueous phase.

TU080

Functions of thioredoxin1 in brain development and in response to environmental chemicals in zebrafish embryos

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Thioredoxin is an evolutionarily conserved antioxidant protein and plays a crucial role for fundamental cellular processes including metabolism, proliferation, differentiation and apoptosis. Growing evidence supports that Thioredoxin has essential functions in the embryonic development and has an influence on cellular response to chemicals insults, particularly those accompanying oxidative stress. We report here that *thioredoxin1* becomes differentially expressed in zebrafish embryos after the exposure to 9 out of 11 environmental toxicants. Changes in *thioredoxin1* mRNA levels were observed at low chemicals concentrations that did not cause morphological defects. *In situ* gene expression analysis showed that *thioredoxin1* was expressed in neurons such as Mauthner and serotonergic Raphe cells, olfactory epithelia, liver and swim bladder under normal conditions. After methylmercury exposure, however, *thioredoxin1* was ectopically induced in the hair cells of the lateral line and in epithelia of the pharynx. Knockdown of thioredoxin1 by antisense morpholinos induced hydrocephalus, concomitant with apoptosis in the brain ventricular epithelium, and exacerbated the toxic effects induced by methylmercury exposure. This study represents the association between hydrocephalus and Thioredoxin1 malfunction in embryonic development and provides valuable information to elucidate the protective role of Thioredoxin1 against chemicals disruption.

TU081

Effects of metazachlor and their main metabolite metazachlor OA on early life stages of marbled crayfish (Procambarus fallax)

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Growing production of energy crops (mainly maize and rapeseed) in Europe, replacing traditional crops such as grain, have significantly changed patterns of pesticides utilization during the last decades. Chloroacetanilide herbicides are a relatively old class of preemergence herbicides for selective weed control in various field crops. The relatively high water solubility of these chemicals makes them potentially mobile in soil and water. Contamination of aquatic ecosystem by herbicides has become a pressing environmental problem all over the world. The effect of early life stages of marbled crayfish (*Procambarus fallax*) to chronic exposure of metazachlor in concentrations 3.2 μg/L, 22 μg/L (max. real environmental concentration), and metazachlor OA in concentrations 3.2 μg/L (real concentration), and 22 μg/L was evaluated under laboratory conditions. The effects were assessed on the basis of mortality, behavior, growth, development, oxidative stress, antioxidant biomarkers and histopathology. Metazachlor and metazachlor OA in all tested concentrations caused significantly higher mortality, lower growth, delay in ontogenetic development, increased of total distance moved and walking speed of crayfish. Moreover, both tested substances in all concentrations caused changes in antioxidant biomarkers (decrease activity of superoxide dismutase, catalase and glutathione s-transferase) and caused alteration of hepatopancreas and gills of crayfish. In conclusion, this study shows potential risk of metazachlor and their main metabolite metazachlor OA on early life stages of crayfish. *The study was financially supported by the MSMT projects CENAKVA (No. CZ.1.05/2.1.00/01.0024) and CENAKVA II (No. LO1205 under the NPU I program) and project GACR no. 18-03712S.*

TU082

Physiological responses of zebrafish Danio rerio to emerging pollutants

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Emerging Contaminants are chemical, pharmaceutical and mainly personal care products, which have been found in concentrations of 0.001 to 500 mg in the waters of treatment plants and in natural systems. There is limited information of the effects of these pollutants on the organisms that inhabit the aquatic systems, for this reason, in this study the effects in physiological rates and biomarkers, (oxidative stress, neurotoxicity and genotoxicity) of 2 liquid detergents (Ariel and Mas Color) and 2 softeners for clothes (Downy and Suavitel) were evaluated in the zebrafish *Danio rerio*. Bioassays were carried out with a duration of 15 days where zebrafish adults were exposed to a sublethal concentration (1 mg L^{-1}) of detergents and softeners. At the end of the exposure period, the growth rate, the respiration rate and the O: N index were evaluated. The degree of lipoperoxidation (Tbars), the activity of the enzyme Acetylcholinesterase (AchE) and the genetic damage (frequency of micronuclei) were also determined. In the results obtained it was observed that there are significant differences in the physiological rates of the exposed organisms compared to the control group. The growth rate of organisms exposed to detergents was the lowest. The values of the O:N index varied from 25 to 8 in the fish exposed to detergents and softeners. The most toxic products were the Ariel detergent and the Suavitel softener, the same products were the highest oxidizing effect (4.54 and 3.13 nM Tbars mg^{-1} respectively). In the tests with detergents a decrease of between 12 to 27% of the activity of the enzyme AchE was observed. In the evaluation of genotoxicity, a greater number of micronuclei was observed in the fish exposed to the Ariel detergent (8.2%) and in the Suavitel product (4.1%). The results indicated that all tested products caused deleterious effects on fish in sublethal concentrations.

TU083

Long-term effect of S-metolachlor and metolachlor OA on marbled crayfish
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Various pesticides are being used intensively in agriculture and affect almost every system of environment especially aquatic ecosystems and causing risk hazards for aquatic flora and fauna, as well as their residues. They enter in non-targeted animals via food chain threatening the ecological balance and biodiversity of the nature. Long-term exposure of pesticides can induces behaviour, biochemical changes, oxidative stress, histopathological damages, etc. This study evaluates selected parameters during long-term effects of S-metolachlor (S-M) and its metabolite metolachlor OA (M-OA) at concentrations of 4.2 μg/L (environmental concentrations) and 42 μg/L (ten times higher environmental concentrations) for each of them on marbled crayfish (*Procambarus fallax* f. *virginalis*). The samples of crayfish were sampled on day 14, 28 exposure and after another 28-day for recovery period in S-M and M-OA-free water under laboratory conditions. This study shows that 14 and 28 days exposure to S-M and M-OA caused significant changes ($P < 0.01$) in biochemical haemolymph profile (lactate, alanine aminotransferase, aspartate aminotransferase, inorganic phosphate); lipid peroxidation in hepatopancreas; affected antioxidant parameters (catalase, reduced glutathione, glutathione S-transferase) of hepatopancreas and gill which have not been stabilized even after 28 days of recovery of crayfish. As well as rapid lower movement and activity, and histopathology damage of gills and hepatopancreas due to exposure were persisted in crayfish even after being kept in recovery period 28 days respect to control. The data obtained provides a number of data on the harmful effects of S-M and metabolite M-OA on non-target organisms, naturally occurring in the environment. And it provides further information for assessing the hazards of these substances. *The study was financially supported by the MSMT projects CENAKVA (No. CZ.1.05/2.1.00/01.0024) and CENAKVA II (No. LO1205 under the NPU I program), project GACR no. 18-03712S, and project Development of University of South Bohemia: International Mobility MSCA IF (No. CZ.02.2.69/0.0/0.0/17_050/0008486).*

TU084
Identification of molecular mechanisms behind increased locomotor response in zebrafish larvae exposed to a human relevant POPs mixture and PFOS.
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Previously, we observed behavioural effects in zebrafish larvae exposed to a mixture of persistent organic pollutants (POPs) based on average blood levels from the Scandinavian population. This POPs mixture contains 29 chlorinated, brominated and perfluorinated compounds and we identified PFOS as the compound driving the behavioural changes. To further understand the molecular mechanisms behind these effects we exposed zebrafish larvae from the blastula stage until 96 hpf to the POPs mixture or PFOS only. We validated our previous results by performing locomotor behavioural tests on 96 hpf larvae that were exposed to two levels (10x and 70x higher than what is found in human blood), to either the POPs mixture or PFOS only. In order to identify molecular mechanisms we performed RNA sequencing analysis on 96 hpf larvae. Results showed that larvae exposed for 96 hours to the highest concentrations of POPs and PFOS showed hyperactivity in terms of swimming speed. Transcriptomic analysis showed upregulation of genes such as myh4, myh7, myl7, tnnc1, tnni2 and tnnt2, which play a functional role in muscle contraction. Muscle contraction is highly regulated by the availability of calcium in the sarcoplasmic reticulum. Ingenuity pathway analysis showed that one of the affected pathways in larvae exposed to the POPs mixture and PFOS was calcium signaling via the activation of the ryanodine (RyR) receptors. Previous studies have shown that POPs bind and activate different ryanodine receptor (RyR) isoforms increasing the calcium release. Thus, the observed hyperactivity of larval zebrafish we observed in the locomotor test might be linked to increased availability of calcium, which leads to increased muscle contraction. Functional studies with Ryr inhibitors will be performed to substantiate these findings. We also found effect on lipid metabolism in those larvae exposed to the lower concentration of PFOS. Affected pathways indicate downregulation of lipid metabolism in terms of synthesis of lipid, fatty acid metabolism and lower uptake of lipid among others. Lipidomics analysis is being carried out to investigate the effects of POPs mixture and PFOS single exposure on the lipid contents of larval zebrafish. By using omics technology, we observed that the altered behavioral pattern in exposed zebrafish larvae seems to be controlled directly by mechanisms affecting muscle function rather than from mechanisms connected to neurotoxicity.

TU085
Effect directed analysis (EDA) of neuroactive substances in WWTP effluent using Danio rerio embryos and behavioural assays
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In the last years, neuroactive compounds became an emerging issue for human and aquatic life. In a recent study on EU rivers basins, 13% of the detected compounds were classified as neuroactive making neurotoxicity one of the major modes of action (MoA) identified in environmental samples. Despite their potential threat for the aquatic ecosystem, there is still lack of methodologies for the identification of neurotoxicity in environmental samples. Moreover, chemicals are normally present in complex mixtures in the environment resulting in unpredictable outcomes in case of their interactions. However, effect directed analysis (EDA) with a combination of fractionation, chemical analysis and bio testing can identify masking effects and reveal the presence of primarily causative chemicals. In this study, we proposed a workflow for the identification of potential neuroactive substances using *Danio rerio* embryos in a 96 hours test. Water extracts were collected downstream of the waste water treatment plant (WWTP) of Bitterfeld (Germany) using on-site large volume solid phase extraction (LV SPE). Chemical analyses were performed using an LC-RMS with a wide list of possible active target chemicals (580). The sample was subsequently fractionated using a reversed phase semi-preparative C18 column and 20 fractions were collected. In order to identify potentially neuroactive substances, we analysed the embryos behavioural pattern under light/dark conditions. Moreover, we analysed the inhibition of the acetylcholinesterase (AchE) to identify possible organophosphates and carbamates. Preliminary results confirmed the presence of 21 neuroactive chemicals. The 96 hours old embryos showed an increase of the light sensitivity at low concentrations (relative enrichment factor (REF) of 1) followed by a progressive decrease of the locomotion in parallel with increasing concentrations. Moreover, we identified 3 active fractions characterized by hyperactivity and hypoactivity. A common loop diuretic (furosemide) was detected in the fraction which caused hyperactivity while the antidepressant amitriptyline was detected in the fraction generating hypoactivity. Overall, preliminary results confirmed the suitability of Danio rerio embryos for the identification of neuroactive compounds in complex mixtures.

TU086
Assessment of neurotoxicity and oxidative-stress markers in seabass exposed to emerging compounds
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Nanomaterials and a wide-range of biocides have been regarded as emerging contaminants receiving a remarkable scientific attention in the last decade, particularly in terms of their sub-cellular effects. Biomarkers of effect indicate an early biochemical response that has occurred following exposure of an organism to a contaminant. Oxidative stress and neurotoxicity play an important role in the intracellular biochemical imbalance that can ultimately lead to cellular death. For instance, increase of lipid peroxidation (LPO) levels can indicate oxidative degradation of cell membrane lipids caused by free radicals and reactive oxygen species; changes in enzymatic activities (e.g. catalase (CAT) or glutathione-S-transferases (GSTs) can signalize an impairment of the antioxidant system or in regular biotransformation intracellular mechanisms; inhibition of acetylcholinesterase activity indicates a neurotransmission impairment. The aim of this study was to assess biochemical changes of new or emerging contaminants such as nanostructured materials and biopesticides in marine fish, at sub lethal levels. Juvenile *Dicentrarchus labrax* were exposed to the biopesticides (*Quillaja* saponins at 0.017-4 mg/L), unloaded anionic nanoclays (Zn-Al layered double hydroxides; LDH at 0.11-9 mg/L) and a novel protein adsorption inhibitor (0.03-7.3 mg/L). Individualswere sub-chronically exposed to each chemical for 14 days,following the OECD guideline 204, under semi-static conditions. Range-finding tests were previously performed using acute toxicity tests with *D. labrax* or with embryos of *Sparus aurata.* At the end of the exposure liver samples were used to spectrophotometrically determine protein and LPO levels and to measure GSTs, CAT and AChE activities. Exposure to *Quillaja* saponins and the protein adsorption inhibitor induced significant biochemical changes, however no neurotoxic effects were observed. Saponins induced oxidative stress and activated the detoxification system (LOEC=1.3 mg/L) while CAT was inhibited at lower exposure concentrations (LOEC=0.4 mg/L). Very low exposure concentrations of protein adsorption inhibitor induced oxidative stress and inhibited CAT (LOEC=0.09 mg/L) and promoted an increase of GST at 0.03 mg/L. Finally, Zn-Al LDH exposure caused no significant changes in the tested endpoints. In conclusion, this research confirms that the quantification of biomarkers of effect is a good tool to assess toxicity effects at sublethal levels of chemicals.

TU087
Investigating the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on sex differentiation in zebrafish
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Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively used for their analgesic and anti-inflammatory properties. Due to their high usage, they are continuously being released into the environment. NSAIDs can be toxic even at low concentrations and many of their effects are thought to result from their ability to inhibit cyclooxygenase enzymes (COX-1 and/or COX-2) that causes decreased synthesis of prostaglandins. Prostaglandins, in turn, are involved in several physiological functions, such as cell survival, apoptosis and regulation of the mammalian sexual behavior. The aim of the present study was to examine the possible effects of NSAIDs on zebrafish gonad differentiation, since NSAIDs inhibit the rate-limiting cyclooxygenases enzymes in prostaglandin synthesis. The NSAIDs in the present study were selected based on their COX selectivity (COX-1 selective: acetylsalicylic acid, ketoprofen; COX-2 selective: nimesulide, celecoxib; non-selective: indomethacin, naproxen, ibuprofen). Wild-type and the vas::EGFP transgene juvenile zebrafish were exposed to 100 μg/L NSAIDs in crystallization dishes with 100 mL final volume. Fifteen- and 20-dpf zebrafish were exposed to NSAIDs for 6 days. Thereafter the fish were collected, sacrificed by snap-freezing in liquid nitrogen and stored at - 80 °C for further use in gene expression analyses. To determine if the NSAIDs altered the sex ratios, 15-dpf fish were exposed until 40-dpf and transferred back to the circulating system at the end of exposure. The sex ratios were thereafter determined following dissection and observation of the gonads under a fluorescent microscope at 70 dpf for the vas::EGFP transgene and at 90 dpf for wild-type zebrafish. Preliminary sex ratio data showed that exposure to indomethacin, naproxen, and nimesulide resulted in significantly increased masculinization of zebrafish. Moreover, gene expression analysis at 26-dpf showed decreased expression of prostaglandin synthases *ptgs1*, *ptgs2a* and *ptgs2b* and the female-specific genes *zp2*, *vtg2* and *foxl2.* The results are in agreement with our earlier studies with meloxicam, supporting that NSAID exposure results in male-biased sex ratios. This study will provide detailed information on expression profiles of several sex related genes and pathways, and reveal the mode of action of NSAIDs on the sex differentiation in zebrafish.

TU088

How critical is xenoestrogen exposure during development for immunocompetence of fish?

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The farming of fish inflicts various stresses upon the animals, which may impact their immune system. A reduced capacity to fight pathogenic agents may entail important losses in aquaculture production. Oestrogens are well-known endocrine disruptors that are present in natural forms in fish farms because non-degraded hormones, such as oestradiol, are released by the fish into the water body. Oestradiol is involved in the regulation of numerous physiological functions, including the modulation of immune system function and performance. Hence, we studied the long-term consequences of exposure to environmentally relevant concentrations of oestradiol on fish immunocompetence. Fingerlings of 47 and 60 days post-hatch were exposed to 20ng/L of oestradiol for 7 days and one month, respectively. Water samples were collected and analyzed by radioimmunoassays. Following exposure, each stage was sampled for gene expression analysis to assess the impacts of oestradiol on the ontogenesis of the thymus, which is the primary lymphoid organ responsible for T cell differentiation. After depuration phase, the fish were challenged by intracoelomic injection with *Vibrio harveyi* and cumulative mortality was recorded. Exposure to oestradiol, which was detected in the exposure water, from 47 to 54 days post-hatch caused an increase in sea bass mortality following bacterial challenge. By contrast, oestradiol exposure from 60 to 90 days post-hatch improved the fish resistance to vibriosis. As in mammals, oestradiol seems to have immunostimulating or immunosuppressive effects depending on the critical window of development. The relation between these immunomodulatory effects, thymus development and associated peripheral T cells is currently investigated.

TU089

Landscape Endocrinology: Wastewater Reuse, Exposure Risk, and Fish Endocrine Disruption

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Reuse of municipal wastewater treatment plant (WWTP) effluent is an important component in augmenting global freshwater supplies. One of the challenges associated with reusing municipal wastewater is the presence of biologically active contaminants, such as endocrine-disrupting compounds (EDCs), which not only influence human consumption of reused wastewater but also impacts ecosystems which provide hydrologic connectivity between WWTP discharge locations and intakes for drinking water treatment plants. Risk assessment for potential endocrine disruption requires a landscape-based approach that allows investigations from river reach to continental scales. Widespread fish endocrine disruption, in the form of intersex and elevated plasma vitellogenin in male smallmouth bass, has been reported in the Shenandoah River Watershed (SRW). Detailed studies were conducted on the relationship between EDC exposure pathways and biological endocrine disruption effects incorporating wastewater reuse modeling, field sampling of water chemistry, and on-site fish exposure experiments. The amount of WWTP effluent in each river reach (accumulated wastewater ratio -ACCWRatio) was determined and used to predict environmental concentrations for select WWTP derived EDCs, including the steroidal estrogens estrone, 17-β-estradiol, estriol, and 17-α-ethinylestradiol. Mobile laboratory fish exposure and water characterization experiments were conducted at 9 locations to assess different source waters for the presence of EDCs and fish endocrine disruption using vitellogenin induction in male fathead minnows as the biomarker. Although the various source waters had complex mixtures of chemicals, the measured and predicted environmental concentrations of select steroidal estrogens resulted in 17-β-estradiol equivalency quotients ranging from < 0.5 to 5 ng L^{-1}, indicating low-to-moderate risk of fish endocrine disruption. Results from the fish exposure experiments showed minimal estrogenic effects as indicated by the low vitellogenin induction in male fathead minnows.

TU090

Thyroid hormone disrupting effects of two UV filter agents (EHMC and BP-3) in zebrafish embryo-larvae and ZFL (zebrafish liver cell line)

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During the last decades, the use of UV filters including 2-ethylhexyl 4-methoxycinnamate (EHMC) and 2-hydroxy-4-methoxybenzophenone (BP-3) has been significantly increased. Widespread use of EHMC and BP-3 has led to frequent occurrences of these compounds in the water worldwide. Previous studies have reported sex hormonal activities of EHMC and BP-3. However, there are knowledge gaps in thyroid hormone disruption of these chemicals in aquatic organisms. In this study, we investigated thyroid disrupting effects of EHMC and BP-3 in the embryo-larval stage of zebrafish and possible mechanisms by using zebrafish liver cells (ZFL). The zebrafish embryos were exposed to 1-30 μM of EHMC or 0.14-1.40 μM of BP-3 for 6 days. Embryo and larval survival, hatchability, malformation rate, and thyroid transcription level were observed. In ZFL experiment, cells were exposed to 1-10 μM of EHMC or 3-30 μM BP-3. Exposure to EHMC or BP-3 significantly altered thyroid hormone systems of zebrafish larvae and ZFL. No significant changes of the embryo and larval survival, hatchability, and malformation rate have resulted following exposure to EHMC, except decreased hatchability observed at 1.40 μM of BP-3.
For *crhb*(≥1μM) and *tg*(>1μM), gene expression was significantly down-regulated ($p< 0.05$). Also, fold change of *tshb*, *tshr*, *nkx2.1*, *ugt1ab*, *sult1st5*, *dio1*, *dio2* and *ttr* were decreased in zebrafish larvae exposed to EHMC. Moreover, *tg*, *ugt1ab* and *dio1*were significantly up-regulated ($p< 0.05$) at 1.40 μM, and fold change of *tshb*, *tpo*, *nis*, *pax8*, *dio2* and *ttr* mRNA expressions were increased in zebrafish larvae following exposure to BP-3. In ZFL exposure, increased regulation of *ugt1ab*(>1.5 fold) was observed, but no genes were changed significantly. Following exposure to BP-3, significant ($p< 0.05$) decrease of *ppara*(≥3 μM) and dose-dependent up-regulation of *ugt1ab*(>1.5 fold) gene were observed in the liver cells. Our observations suggest that exposure to two commonly used sunscreen chemicals could disrupt normal thyroid function by interfering central regulation, synthesis, and metabolism of the thyroid hormones. A different pattern of thyroid regulation was observed from exposure to EHMC and BP-3. Further studies are needed to confirm different modes of thyroid disruption by these frequently used sunscreens and related health consequences.
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TU091

Potential thyroid disrupting effects of p,p'-DDE in zebrafish larvae, rat pituitary (GH3) and zebrafish liver (ZFL) cell lines

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DDT (dichloro-diphenyl-trichloroethane) was one of the most used organochlorine pesticides for public health and agricultural purpose. Although its use has been banned for over 40 years, this pesticide along with its major metabolite *p,p'*-DDE (dichloro-diphenyl-dichloroethylene) are still detected in the biota. While DDT and its metabolites have been associated with thyroid disruption in epidemiological studies, little is known about the thyroid disrupting effects in aquatic organisms. We investigated adverse effects of *p,p'*-DDE in thyroid system of zebrafish embryo-larvae and possible mechanisms by using a rat pituitary (GH3) and a zebrafish liver (ZFL) cell lines. Zebrafish embryos were exposed to 0.01 to 0.3 µM of *p,p'*-DDE for 144 hours post fertilization and measured for embryo and larval survival, hatchability, malformation rate and transcription of genes related to thyroid hormones. GH3 and ZFL cells were respectively exposed to 1 to 10 µM and 0.3 to 3 µM of *p,p'*-DDE, for 48 hours. Key genes regulating thyroid hormone balance were measured in each cell. In zebrafish, larval survival was significantly decreased (< 80%) at 0.3 µM and the *crhβ*, *tshβ*, *nkx2.1*, *tg*, *sult1 st5*, *dio1* and *dio2* genes were significantly up-regulated at the sublethal concentration of 0.1 µM. Adversely, *Tshβ* mRNA transcription was significantly down-regulated at 10 µM in GH3 cells. In ZFL cells, increased pattern of *ugt1ab* gene was observed while not statistically significant. Our results suggest that exposure to *p,p'*-DDE could affect thyroid hormone balance in developing zebrafish. Both *in vivo* and *in vitro* data showed transcriptional change of the genes related to central regulation, which may impact thyroid hormone synthesis and lead to altered hepatic metabolism of hormones. Future studies are needed to confirm the changes in thyroid hormone levels following exposure and to investigate possible adverse outcomes. **<strong style="font-family: 'times new roman', times; font-size: medium;">[Acknowledgement]** This study is supported by a grant (18182MFDS365) from Korean Ministry of Food and Drug Safety (MFDS) in 2018.

TU092

Effects of exposure to perfluorooctanoic acid (PFOA) on the adult Australian native freshwater fish Melanotaenia fluviatilis

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PFOA has been used to manufacture several products and does not degrade easily in the environment. In this study, the Australian native fish Murray River rainbowfish (*M. fluviatilis*) were exposed to four different concentrations of perfluorooctanoic acid (PFOA) (0.01, 0.1, 1 and 10 mg L^{-1}). Changes in the thyroid hormones T3/T4 ratio, vitellogenin, changes in oxidative stress and lipid peroxidation were determined after 14 days of exposure. Activities of catalase (CAT) and glutathione- S- transferase (GST) were significantly increased in the gills and significantly reduced in the liver. Lipid peroxidation was observed in both tissues showing that vital organs could not neutralize the peroxides generated by oxidative stress resulting from exposure to PFOA. Fish exposed to PFOA had altered T3/T4 ratios and vitellogenin was induced in the plasma of male fish. Disruption of the hormonal balance by exposure to perfluorinate compounds (PFCs) in the environment can lead to negative effects on fish, severely compromising their fitness and survival resulting in and failure to adapt to changing environmental conditions.

TU093

Fish mucous barriers respond significantly to pollutants and parasites in shorthorn sculpins (Myoxocephalus scorpius) from 3 sites near a former lead-zinc mine in Maarmorilik, west Greenland

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Sculpins caught near the former lead – zinc mine in Maarmorilik, West Greenland are well documented as being impacted by heavy metal contaminants. In this study, we applied mucosal mapping, a novel, quick and efficient method for stereologically quantifying mucosal responses in fish, to shorthorn sculpins caught in contaminated areas in Maarmorilik, to investigate interactions between the host, parasites and heavy metal exposure (Pb and Zn). We found that the sculpin's mucosal epithelia in the skin and gills reacted in a quantifiable and reproducible manner to environmental heavy metal exposure and parasites. The skin barrier status was not affected by heavy metals but responded continuously to ectoparasite infestation. Skin mucous cells were significantly smallest at station 3 where the skin parasite load was highest and heavy metal residues were lowest. In the two populations of gill mucous cells (filamental and lamellar), the largest and densest filament mucous cells were found at the most polluted station. The density of gill lamellar mucous cells followed a toxicity gradient and was significantly highest at the most polluted station and significantly lowest at the least polluted station. The persistent presence of toxic Pb and Zn levels in the sediment at Station 1 may have induced a small but measurable reduction in the surface area available for respiration and excretion as a result of chronic exposure to these environmental toxins in the shorthorn sculpin. The strong correlation between size of filament mucous cells and liver concentrations of Pb suggests these cells can play an active role in reducing the somatic load of Pb in sculpin. These mucosal epithelial changes can be used to indicate fish adaptations and differential responses to environmental challenges in field studies.

TU094

Evaluation of toxic effects caused by surfactants used in nano-medicine on the aquatic environment

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Surfactants are used in various fields such as personal care products and detergents. For the production of nano-formulations, surfactants are applied to change surface properties of nano-particles or to stabilize the colloidal system. Such nano-medicines could also enhance the specific accumulation of pharmaceuticals in tumor cells or increase the passage of physiological barriers such as the blood-brain-barrier. However, surfactants could also inhibit enzymes and cause the lysis of cells due to alteration of the cell membrane. Surfactants used in nano-medicine can enter the aquatic environment via production or incorrect disposal where they can cause toxic effects and changes in behavior. Therefore, it is essential to examine possible negative effects of surfactants on the aquatic environment. The aim of this study is thus to identify surfactants which lead to the least negative effects in aquatic organisms. For this, the zebrafish embryo *Danio rerio* is used in the Fish Embryo Toxicity test (zFET). The following surfactants were tested: polyethylen-polypropylen glycole (here: Pluronic® F127 und Pluronic® F 68)**,** polyoxyl-40 hydrogenated castor oil (Cremophor® RH40) and polyethylen-glycol-1100-mono(hexadecyl/octadecyl)-ether (Cremophor® A25). For Cremophor® A25, an EC_{50}-value of 0,001 % was determined at 72 hours post fertilisation (hpf). Cremophor® A25 caused approximately 100 % mortality at a concentration of 0.05 % (72 hpf). At a concentration of 0.5 %, Cremophor® RH40 leads to 100 % mortality (72 hpf) and the EC_{50}-value was 0.007 % (72 hpf). Embryos which were exposed to Pluronic® F127 and Pluronic® F68 displayed sub-lethal deformations of the head, the tail and the chorda. The EC_{50}-values of Pluronic® F68 and Pluronic® F127 were 1.152 % and 0.808 %, respectively, at 72 hpf. Interestingly, the chorion of embryos exposed to Pluronic® F68 and Pluronic® F127 was deformed at the lowest concentration of 1.5 % within a few minutes after the start of exposure. Based on the EC_{50}-values, we can conclude that Cremophor® RH40 and Cremophor® A25 cause one to two times higher toxic effects on aquatic organisms than the group of polyethylen-polypropylen glycole (Pluronic® F68 und Pluronic® F127). From an eco-toxic point of view, the polyethylen-polypropylen glycole are thus more appropriate for the production of nano-medicines. Funded by Deutsche Bundesstif-tung Umwelt (AZ32725)

TU095

Simple energy-budget model for yolk-feeding life stages; a case study for Atlantic cod

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The early life stages of organisms are of considerable interest in ecotoxicology as they constitute a vital aspect of population dynamics and often display considerable sensitivity towards toxicants. Furthermore, embryo and early-life stage toxicity tests for vertebrates (i.e., fish and amphibians) are increasingly being suggested as alternatives for testing with the later (and legally-protected) life stages. To interpret the patterns of effects observed in toxicity test, and to extrapolate such results to field conditions, requires mechanistic models. Such models should consider both the toxicokinetic (TK) and toxicodynamic aspects (TD) of toxicity. Substantial research efforts are currently concentrating on the molecular level. However, we are convinced that molecular-level approaches need to be combined with energy-budget models to allow for a causal link between exposure and the whole-organism life-history traits (as represented by AoPs). The reasons for this are twofold. Firstly, any AoP will require knowledge about TK: how the internal concentration (in specific parts of the organism) develops over time. TK will be influenced by how the various biomass components (e.g., structure and yolk) develop over time, which is served by energy-budget models. Secondly, life-history traits are connected through the energy budget (e.g., structural growth and development are causally linked to the available yolk and to respiration rates), and feedbacks at this level cannot be explained from the molecular level up. Unfortunately, there have so far only been few attempts to apply energy-budget models to yolk-feeding stages. In this contribution, we report on the first stage of our attempts to do so, applying the simple DEBkiss model. In this first stage, we tested the basic model (in absence of toxicant stress) on extensive data for the yolk-feeding stages (and subsequent larval starvation) of Atlantic cod (*Gadus morhua*). The model provided an excellent explanation for the development of total weight, yolk content, respiration and larval length over the development of the egg and the yolk-sac fry. We include model simulations to predict how toxicant stress is expected to affect these development patterns. In a later stage, we will use our model to interpret toxicity data and to attempt a link with gene expression and metabolomics.

Ecotoxicology of Nanoplastics: Mechanistic Approaches to Understand Their Risk for the Environment and Human Health (P)

TU096

Polystyrene microbeads' uptake, tissue distribution and toxicity on zebrafish embryos, and their sorption capability for triclosan

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Few studies related to microplastic (MP) contamination focus on ingestion and effects on freshwater species and even less is known about the potential action as carrier for some aquatic contaminants. The present study aims to investigate the microplastics' ingestion, tissue distribution and toxicity on zebrafish. Embryos were exposed for 48 h to virgin polystyrene microbeads (50 mg/L) with a dimension of 0.5 μm. Further, the possible absorption of chemicals on MPs was evaluated by the quantification of the absorbed concentration of triclosan (TCS), a common used antibacterial agent. MP uptake and tissue distribution were evaluated with the advanced confocal microscopy technique. The potential effects due to the ingestion of MPs were assessed through the analysis of P-glycoprotein (p-gp) transporter efflux and cyclooxygenase (COX) activity, while the oxidative stress was assessed by the analysis of reactive oxygen species (ROS) production, protein carbonyl content (PCC) and the activity of antioxidant/detoxifying enzymes glutathione S-transferase (GST), catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). The adsorption of TCS on MPs was assessed mixing a MP powder in a TCS working solution. To quantified the absorbed concentration of TCS, an aliquot of treated-MPs was extracted in hexane, ultrasonicated and analysed using a high-resolution GC-MS system. Imaging observations confirmed MPs' uptake, distinctly showing embryos with beads inside the gastrointestinal tract and some of them migrating outside the intestinal epithelium. Moreover, some images showed the existance of potential alternative uptake routes, such as neuromasts or gills. The overall biomarker results revealed only a slight toxicity of MPs. Adsorption analysis showed that TCS can adhere to MP surface and this outcome underlined a potential synergistic effect of MPs on aquatic organisms. This study highlights the need of more deepened analyses evaluating the risk associated with MP infiltration and the interaction between microplastics, associated contaminants and organism.

TU097

Quantitative analysis of the impacts of microplastics on the freshwater duckweed Lemna minor

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Microplastic pollution has been widely reported in marine ecosystems all around the world. The intake of microplastics by different marine species has also been described in both wild caught and laboratory exposed samples. However, data on the presence of microplastics in the freshwater environment are scarce and the effects on aquatic species are not clear. In this study, the potential impacts of plastic microbeads on the freshwater plant *Lemna minor* were investigated. The interaction and potential effects of 1 μm polystyrene (PS), 1 μm polyethylene (PE) and 10-45 μm polyethylene (PE) beads on the physiology of the widely distributed freshwater duckweed *Lemna minor* were firstly investigated. The adsorption of 10-45 μm PE beads per surface area was quantified at five time points (3, 24, 72, 168 and 240 h) by using light and fluorescence microscopy. The fate of 1 μm microbeads within the plant tissue was further examined using confocal laser scanning microscopy. In addition, a thorough study on the growth and physiology of *L. minor* following a seven-day exposure to PS or PE microbeads was quantified following OECD parameters: frond and colony number, root length, chlorophyll *a* fluorescence and relative growth rate. Our results indicate that microplastic binding on plant tissue is time-dependent, however, despite the significant strong binding of microbeads to the duckweed surface over time, no negative effects on the physiology of *Lemna minor* were found. The strong microbead binding to duckweed tissue suggested *L. minor* as a potential vector for the transfer of microplastics to invertebrates. These results indicate that although microplastics interact with duckweeds, they do not have an effect on plant physiology, growth or photosynthetic efficiency

TU098

Secondary nanoplastics released from biodegradable microplastics severely affect freshwater organisms

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Despite the recent advances in the field of plastic ecotoxicology, the ecological impact of nanoplastics in the environment remains poorly understood. Here, we have investigated the effects of secondary nanoplastics of polyhydroxybutyrate (PHB), polylactic acid (PLA) and polycaprolactone (PCL), biodegradable plastics, to representative organisms of aquatic ecosystems. Regarding PHB, secondary nanoplastics were produced from PHB microplastics by abiotic degradation under environmentally representative conditions. We have performed a complete physicochemical characterization confirming the presence of secondary PHB-nanoplastics in the 75 – 200 nm range. PHB-nanoplastics induced a significant decrease in cellular growth and altered relevant physiological parameters in all organisms. We investigated whether the observed toxicity was exerted by secondary PHB-nanoplastics themselves or by other abiotic degradation products released from PHB microplastics. An experiment was run in which secondary PHB-nanoplastics were removed by ultrafiltration; the resulting supernatant was not toxic to the organisms, ruling out the presence of toxic chemicals in the PHB microplastics. Results with PLA and PCL are still preliminary but we have also found the formation of a nanometric fraction from abiotic degradation of both polymers, toxicity evaluation is under way. All results put together indicated that secondary nanoplastics released as a consequence of abiotic degradation of microplastics seem to be harmful for aquatic organisms, suggesting that biodegradable plastic does not mean safe for environment. \n *Acknowledgement* - This research was supported by CTM2016-74927-C2-2-R grants from MINECO/FEDER EU and the Dirección General de Universidades e Investigación de la Comunidad de Madrid, Research Network S2013/MAE-2716. MGP thanks the Comunidad de Madrid – EU for the award of a postdoctoral grant. MTB thanks the Spanish Ministerio de Educación y Formación Profesional for the award of a predoctoral grant, thanks the GA, thanks the Universidad de Alcalá for the award of a predoctoral grant. \n

TU099

Chronic toxicity study of two-sized microplastics in marine copepod Tigriopus japonicus

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Microplastic particles are ubiquitous in the environment and are growing concern nowadays. In this experiment we performed chronic exposure of two sized polystyrene microplastics, 50 nm and 2 um, to marine copepod Tigriopus japonicus. Exposure was started from the Nauplius stage and conducted for 30 days to identify the effect on growth, and another 10 days exposure was performed to egg sac bearing adult female to examine the reproductive effect. Mortality of 30 days exposure presented 30 day-LC50 value 0.1 mg/L for 50 nm PS sphere, and 3.9 mg/L for 2 um PS sphere. Another individual level effects on development time (nauplius to copepod, N-C, nauplius to adult, N-A), fecundity (number of clutch, number of nauplii/clutch, hatching period, number of nauplii/10days, hatching failure) was also examined. There was no significant effect on development or fecundity, but female exposed in the 10 mg/L above concentration showed higher hatching failure. For evaluation of molecular level effects, we also measured ROS and NO levels and gene expression changes that related with oxidative stress, inflammation, molting, and reproduction. The molecular effects caused by microplastics were significantly different from the control group.

TU100

Cellular effects of natural and synthetic microparticles in marine invertebrates

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Coastal and estuarine invertebrates are exposed in their habitats to various natural particles, such asłfine sand grains, diatoms shells, cellulose fibres, or chitinous fragments. Additionally, they encounter anthropogenic litter and synthetic micro-particles. Especially the latter are of interest in ecotoxicological research as they were reported to induce cellular stress and, finally, damages of biomolecules. Comparative studies on the cellular effects of natural and synthetic particles are yet lacking. Therefore, we will perform feeding experiments with two ecologically and commercially important invertebrate species from the coastal North Sea. Blue mussels (*Mytilus edulis*) and brown shrimps (*Crangon crangon*) will be collected from the field, acclimated to the laboratory conditions, and exposed to various types of natural and synthetic particles. Incubations will last 6, 24, and 48 hours, respectively. A series of microscopic and histological analysis will localize the ingested particles in the digestive organs. Biochemical and histochemical analyses will be used to measure stress markers in the cells. The goal of this doctoral work is to identify, understand, and to distinguish biological effects of microparticles of anthropogenic and natural origin. The findings will help to better understand and estimate the hazard of microplastics to marine invertebrates and to define their actual threat boundaries._

TU101

Responses of nematodes to microplastic exposure - direct or indirect effects?

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Apart from global warming, microplastic pollution is currently one of the biggest environmental concerns. Since plastic debris is not biodegradable, it accumulates, rather than decomposes in the environment. Secondary microplastics are a result of the breakdown of larger plastic items caused by weathering via mechanical action, oxidative weathering or biological degradation. Microplastics (The aim of this study was to assess the toxicity of polystyrene (PS) beads of various sizes (0.1 to 10 μm) on the reproduction of the nematode *Caenorhabditis elegans*. Direct effects were related to the ability of the nematodes to ingest the PS beads, which is dependent on the bead diameter and the buccal cavity size of *C. elegans*. Moreover, the link of effects to the ratio of food bacteria and PS beads was studied to evaluate the impact of food availability as an indirect toxicity pathway. *C. elegans*ingested PS beads of 0.1, 0.5, 1.0 and 3.0 μm, whereby 6.0 and 10.0 μm were not found in the nematode's intestine.Toxic effects of the tested PS bead sizes on *C. elegans*' reproduction occurred dose-dependently, with EC50-values ranging from 0.2 to 8.8 mg PS beads ml^{-1}. While the toxicity based on concentrations (mg ml^{-1}) decreased with bead diameter, toxicity based on total surface area (cm^2ml^{-1}) was not dependent on bead size (EC50 ranging between 30 and 100 cm^2ml^{-1}), suggesting that the toxicity mechanism is induced by the surface area of the beads, irrespective of their uptake into the nematodes. Nevertheless, neither oxidative stress nor the potential toxic effects of leachates (e.g. the monomer styrene) could explain the toxicity of the PS beads. The reduction of food availability by the presence of PS beads, however, might at least partly explain the inhibited reproduction of *C. elegans*. Therefore, it is advised to consider also indirect food web effects in studies dealing with the ecological risk of microplastics.

TU102

Effects of polystyrene microplastics and two organic pollutants in different life stages of brown trout (Salmo trutta f. fario)

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Plastic debris is ubiquitously distributed in aquatic and terrestrial environments. Microplastics are all plastic items with a size smaller than 5 mm. Despite the number of studies on effects on freshwater organisms having increased during the last years, the risk of microplastics exerted to freshwater organisms remains unclear and more studies about potential effects of microplastics on organisms are needed. The aim of our study is to investigate effects of polystyrene microplastics (cryogenically milled granules, fractionated to < 50 μm), alone and in combination with organic pollutants, in different life stages of brown trout (*Salmo trutta* f. *fario*). We conducted two fish early life stage tests (FELST) according to OECD 212 and investigated heart rate, hatching success and mortality. After resorption of the yolk sac contents by the fish larvae we, additionally, evaluated behaviour, biometric parameters, the oxidative stress level and the activity of acetylcholinesterase. In the first experiment, effects of polystyrene microplastics alone (100, 10 000 and 100 000 particles/L) were assessed. In a second experiment, we examined effects of polystyrene particles (100 000 and 1 000 000 particles/L) alone and in combination with the antidepressant amitriptyline (300 μg/L). Additionally, we performed two experiments with juvenile fish (9 - 11 months old). In the first experiment, trout were exposed to 10 000 particles/L alone and in combination with the pesticide methiocarb (2 mg/L). In a second experiment, juvenile trout were exposed to either 10 000 or 100 000 particles/L, 100 μg/L amitriptyline or a combination of particles and the antidepressant. In these experiments, behaviour, mortality rate, biometric parameters, the oxidative stress level, the induction of the 70 kD stress protein family (Hsp70) and the inhibition of acetylcholinesterase were under investigation. Furthermore, we recorded histopathological effects in gills and liver of trout. Results showed an effect of microplastics at a concentration of 1 000 000 particles/L on the behaviour of brown trout larvae. Apart from that, microplastics failed to exert any effect or any modulation of methiocarb or amitriptyline effects in different life stages of brown trout. Further analyses are in progress. The present study is part of the joint research project "MiWa" (microplastics in freshwater systems) funded by the German Federal Ministry of Education and Research (no. 02WRS1378).

TU103

Effects of microfibers and tyre debris on freshwater invertebrates

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The majority of studies assessing the toxicity of microplastics (MPs) have focused on the effects of microbeads on marine species. Recent monitoring studies indicate that there is a huge variety of shapes and types of MPs in freshwater and marine ecosystems and that microbeads are not necessarily the most abundant nor problematic ones. Synthetic fibres and tyre debris are emitted in large quantities into the environment and are likely to cause deleterious physical effects due to their elongated or irregular shape. The effects of these MP particles on aquatic organisms, however, are largely unknown. This study aimed to determine the acute and chronic effects of PET fibres and tyre particles on freshwater invertebrate species with different habitat preferences and feeding strategies (i.e., *Daphnia magna*, *Asellus aquaticus*, *Hyalella azteca* and *Lumbriculus variegatus*). Fibres of a length between 101 and 2194 μm (IQR: 493-992 μm) and a width of 29 μm were produced through washing polyester (PET) fleece blankets. Tyre debris was milled from end-of-life passenger tyres (Genan, Denmark) and sieved to obtain a final particle size between 25 and 75 μm. Acute and chronic toxicity studies were carried out based on respective standard protocols (OECD 202/211/225 and ASTM E1706-05). According to their different habitats, the test organisms were exposed to MPs in the water phase (*D. magna and H. azteca*) or mixed into sediment (*A. aquaticus, H. azteca* and *L. variegatus*). A range of concentrations was tested, including environmental relevant concentrations (water exposure: 0, 0.00015, 0.0015, 0.015, 0.15 g/L; sediment exposure: 0, 0.002, 0.02, 0.2, 2 g/kg). For acute exposure, ingestion and immobility or mortality were recorded, while for chronic exposure also reproduction and growth were assessed. All test organisms ingested fibres and tyres, except for *D. magna* which only ingested tyre debris. For the considered endpoints, no adverse effects at the tested MP concentrations were observed, except for *D. magna* reproduction, which was significantly reduced (51%, $p < 0.05$) at the highest test concentration (0.15 g/L). These findings indicate that although freshwater organisms ingest fibres and tyre debris, they do not accumulate within the organisms and do not seem to negatively influence their survival, growth or reproduction at environmentally relevant concentrations.

TU104

The translocation of microplastics to lipid droplets of Daphnia magna is an artefact

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Micro- and nanoplastic particles have been reported to cross the biological barriers of the digestive tract and translocate to other tissues in a number of species. They then could cause inflammatory reactions, making this a relevant topic of investigation. Most prominently, Rosenkranz et al. (2009) described the translocation of 20 nm and 1000 nm polystyrene particles to the lipid droplets of *Daphnia magna.* These findings lack biological plausibility due to the peritrophic membrane in arthropods that would prevent particles larger than 300 nm from interacting with the epithelial layer. The aim of our study therefore was to replicate these findings while covering additional experimental scenarios. We exposed *Daphnia magna* neonates to the same particle types (fluorescent polystyrene spheres of 20 nm and 1000 nm diameter) at two concentrations (2 μg/L and 2 mg/L) for two time periods (4 h and 24 h). Additionally, we adapted a tissue clearing method to the use with *Daphnia* to improve visibility of particles inside the specimen. We used confocal laser scanning microscopy to investigate the translocation of particles from the gut to other tissues, including lipid storage droplets. This is supplemented by experiments regarding the leaching of the dye from the particles. At the higher particle concentration we detected fluorescence in the lipid droplets for both particle types. At least for the 1000 nm particles fluorescence was clearly separate from particles. Our findings suggest that the tissue translocation to the lipid storage droplets reported previously is an experimental artefact caused by the leaching of the dye. This is supported by additional experiments regarding the conditions under which the dye could leach from the particles. This has implications for other studies since fluorescently labelled, commercially available plastic beads are the main method to investigate tissue translocation in aquatic organisms. Additionally it underlines the importance of questioning biological plausibility even in long-standing research.

TU105

Uptake and effects of polystyrene nanoparticles on the early development and life cycle of Tisbe battagliai: role of surface functionalisation

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Plastics are prolific environmental contaminants, widely identified in marine and freshwater ecosystems. Progressive fragmentation of larger plastics leads to the formation of smaller-sized particles whose further degradation will likely result in the formation of plastic particles in the nanoscale (< 100 nm according to nanoparticle definition) with altered physicochemical characteristics that could differ greatly from the original material. Currently there is limited knowledge on the effects of nano-sized plastics, their fate, behaviour and interaction with cellular membranes and organisms. The aim of this study is to better understand the uptake and effects of polystyrene plastic particles in the nanoscale and assess the uptake, bioaccumulation and subsequent developmental and life cycle effects on the marine harpacticoid copepod *Tisbe battagliai*. In this study, the effects on the naupliiar development of *T. battagliai* were assessed over a 6-day exposure period. The mortality and developmental stage of the organisms was assessed daily. At the end of the exposure period, the animals were transferred to clean seawater and the development of the copepodids, adult survival and reproduction was further assessed (assay was terminated on day 21). Due to the current analytical challenges of plastic nanoparticle detection in the environment, polystyrene NPs were used as model particles. Initial investigations focussed on the study of aminated and carboxylated polystyrene particles (PS, PS-NH_2, PS-COOH, 50 nm, Sigma Aldrich and Phosphorex) to assess the impact of surface functionalisation. Characterization of the particles in seawater was performed with dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). Moreover, potential nanoplastic uptake, internationalisation and intracellular localisation is evaluated with confocal microscopy and flow cytometry. Surface-dependent effects were observed, both PS-NH_2 and PS-COOH affected the early life development of *T. battagliai* while no effects were observed for the non-functionalised PS particles. A decreased developmental rate was observed at 5 mg/L PS-NH_2 (88% compared to control) and 10 mg/L PS-COOH (73% compared to control). The number of organisms developing to copepodids was decreased significantly (EC_{50} of 4.4 mg/L and 11.4 mg/L for PS-NH_2 and PS-COOH, respectively). Ongoing studies focus on mechanistic understanding of the observed effects and increasing environmental relevance of the exposures.

TU106

Characterization of cell response in Rhodomonas salina exposed to PMMA nanoplastics

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Due to their small size (< 100 nm), nanoplastics (NPLs) possess specific properties which can potentiate their toxicity towards aquatic organisms depending on surface characteristics and interactions with the surrounding environment. Still, their presence and behavior in the aquatic environment, as well as any toxic mechanisms are, to a large extent, unknown. As primary producers, microalgae play an important role in the functioning of marine ecosystems, producing oxygen and energy and recycling nutrients on which many organisms within the ecosystem rely on. For this reason, negative effects of NPLs in microalgal species will likely have an impact not only on the algae themselves, but potentially also impact other levels of the aquatic food chain. With this in mind, the main objective of this study was to evaluate the cell response of the marine microalgae *Rhodomonas salina* upon 72 hours exposure to plain and carboxylated PMMA (PMMA and PMMA-COOH, 50 nm). Analysed endpoints included growth rate, natural pigments content, cell size, cell complexity, cell viability and cell cycle, reactive oxygen species (ROS) formation, mitochondrial membrane potential, lipid peroxidation (LPO) and DNA content. Additionally, photosystem II (PSII) performance was analysed by PAM fluorometry, to provide further information on the absorption, distribution and use of energy in photosynthesis. PMMA behaviour in exposure media was evaluated using dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). Results obtained showed a different behaviour of PMMA NPLs in exposure media over time, with PMMA forming micro-scale aggregates (2266 ±260 nm) while PMMA-COOH maintained its nominal size range (57.1 ± 0.4 nm). Several differences were seen in terms of toxicity between both PMMA NPLs. PMMA exposure caused a significant effect in cell viability, ROS formation, LPO, pigment content and photosynthetic performance in exposed *R. salina*, probably associated with particle interaction with the cellular membrane. On the other hand, a higher impact of PMMA-COOH was observed in terms of algal growth, photosynthetic performance, cell viability and metabolic activity, with negligible effects seen for ROS formation and pigment content. Overall, surface chemistry and size seem to be key parameters for the interaction and impact of PMMA NPLs in microalgae, and future experiments focusing on the in-depth characterization of the mode of action of these particles are underway.

TU107

Chronic exposure to nanoplastics results in developmental and reproductive toxicities in Caenorhabditis elegans

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Scope: Plastic debris is an emergent global environmental issue. The ultimate product of plastic debris is nanoplastics which are plastic particles less than 100 nm in size that are of particular concern. Due to their enormous quantity, recalcitrant nature, and unique physicochemical properties, nanoplastics exposure to organisms might be chronic posing potential toxicity risk to the ecosystem. **Methods:** We investigated chronic exposure to nanoparticles on the developmental and reproductive toxicities by comparing three types of polystyrene nanoparticles with its: pristine form (NPs-bulk), carboxyl-modified form (NPs-COOH), and amino-modified form (NPs-NH_2) in the nematode *Caenorhabditis elegans*. **Results**: The results showed that all three forms of nanoparticles in moderately hard reconstituted water (EPA water) have a much smaller hydrodynamic diameter than that in K medium. The NPs-bulk did not induce growth defects at the examined concentrations (1, 10, 50 mg/L in EPA water) in *C. elegans*. However, both NPs-COOH and NPs-NH_2 caused a significantly decreased body length in *C. elegans* at 10 and 50 mg/L in EPA water. The NPs-NH_2 did not cause reproductive toxicity in *C. elegans* at the examined concentrations (1, 10, 50 mg/L in EPA water), whereas the total brood size in *C. elegans* was reduced in NPs-bulk and NPs-COOH (50 mg/L for NPs-bulk and 10, 50 mg/L for NPs-COOH, respectively, in EPA water). **Conclusions**: Our results provided evidences that chronic exposure to nanoparticles results in developmental and reproductive toxicities in *C. elegans* and different modified forms of nanoparticles might exert varied degree of toxicity.

TU108

Ecotoxicity of carboxylated nano-polystyrene from laboratory exposures to freshwater organisms

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If plastic pollution in the marine environment is now recognized by scientists as a threat to the balance of its ecosystems in terms of abundance, composition, sources and impact in the ocean, it is then surprising to note that plastic pollution in inland waters is largely unknown. Although the sources, nature, concentration, presence, fate and effects of micro- and nano-plastics have not been well characterized in freshwater systems to date, evidence from the marine environment suggests that micro- and nano-plastics could be considered as emerging contaminants. The production sources and almost all the life cycle of plastics are mainly continental (atmospheric, plastic waste, landfills, agricultural plastics, production and recycling sites, etc.). Thus, for the majority of them, they must transit through the terrestrial and/or fresh water compartment, before reaching the oceanic gyres, where they concentrate, to form the so called “7th continent”. \n In this context, the objective of this work is to provide new data to acquire the knowledge bases, in terms of hazards on freshwater ecosystems exposed to this type of contaminant, which is a prerequisite for a realistic assessment of the risks associated with the presence of plastic debris in our environment. As an example, the potential ecotoxicological effects of commercial dispersions of carboxyled nano-polystyrene (PS-COOH) of 50 and 350 nm in diameter (from 0.1 to 100 μg/L) was studied from laboratory exposures to different freshwater organisms selected for their ecological representativeness (primary and secondary producers *i.e.* different species of microalgae, and consumers *i.e.* larvae of chironomids and amphibian filter-feeders larvae) and for their use as ecotoxicological bioassays. In order to implement the tools necessary to acquire this knowledge, dispersions of PS-COOH were also characterized. Results showed (i) neither mortality nor genotoxicity but a light significant growth inhibition of the size of amphibian larvae exposed to PS-COOH of 50 nm whatever the concentration and to 10 and 100μg/L of PS-COOH of 350 nm, (ii) no effects in terms of mortality, growth inhibition and teratogenicity in chironomus larvae exposed to both PS-COOH, (iii) a significant growth inhibition to both PS-COOH depending on the exposed algae species. Results were obtained in the framework of the French National Program for Environmental and Occupational Health of Anses (EST/2017/1/219), EMPEC.

TU109

Food transfer and uptake of micro- and nanoplastic particles in a two-species marine system

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Research has shown that smaller size plastic particles (< 100nm) are increasingly available for biological uptake on a cellular level. In this context, present study investigates the food transfer of polystyrene particles in two sizes (2μm and 50nm) in a maritime set up with key species *Mytilus edulis* and *Carcinus maenas* of Roskilde Fjord, Denmark. The study was conducted in order to assess the importance of food as source for plastic particles (microplastic (MP) and nanoplastic (NP)) and to what extent the size of particles influenced translocation over biological barriers in the digestive systems of both organisms and thus will be detectable in tissue apart from the stomach such as haemolymph, hepatopancreas and gills. \n Seventy *M. edulis* were exposed through their diet to two concentrations, 500 mg/L and 50 mg/L, of polystyrene particles in two sizes, 2μm and 50nm. The fluorescent particles, coated with an amino function to hinder aggregation, were added to 300 ml artificial seawater (ASW) for each mussels along with algae *Rhodamonas salina* for optimal filtration efficiency for 1h after 30 min depuration. Subsequently, 35 females of the system top predator *C. maenas* were given 1h to acclimate to 1 L ASW and were then left to feed on one *M. edulis* apiece for 1h. After the treatment the 20 crabs and 20 mussels fed with exposed mussels, along with 10 crabs plus 10 mussels as positive controls and 10 negative controls were sampled for detection of micro- and nanoparticles. Four tissue samples for the crabs, (1) gills, (2) hepatopancreas, (3) hemolymph and (4) stomach), and three for the mussels, (1) gills, (2) stomach and (3) the remains, were analysed by fluorescence measurements in a microplate reader and confocal microscopy to determine the presence of the fluorescent polystyrene particles.

TU110

Transfer of Chlorpyrifos from microplastics alters locomotor activity in larval zebrafish

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Microplastics (MP) have become ubiquitously distributed in aquatic environments and have been speculated to cause adverse effects on organisms *via* the transfer of potentially adsorbed pollutants. In the present study, the behavior of zebrafish larvae, which has been proposed as a sensitive tool to estimate the impact of chemicals on physiological and neuronal integrity, was assessed in response to microplastics loaded with the acetylcholine esterase inhibitor chlorpyrifos (CPF; log K_{ow} = 4.96). Embryos at the age of 2 hours were exposed to high-density polyethylene particles (HDPE; 100 particles/ml, 4-6 μm) for 96 hours, which had been incubated in either 0.5 or 5 mg/L CPF solution plus 0.1 % DMSO, respectively. Swimming activity of embryos was detected via video tracking in a light/dark transition assay. Individuals exposed to non-loaded (virgin) microplastics did not show any significant difference in behavior compared to controls, whereas individuals exposed to MP incubated with 5 mg/L CPF showed significantly reduced activity during the dark phases; embryos treated with MP + 0.5 mg/L CPF showed no effects on behavior. The reduction in activity in the MP + 5 mg/L CPF treatment was similar in magnitude to the effects seen in individuals exposed to the positive control (water-borne 0.05 mg/L CPF). Therefore, the HDPE particles may be assumed to have ± quantitatively adsorbed CPF, which was then made bioavailable to the zebrafish embryos. Thus, the present study provides evidence that video-tracking methods for the detection of behavioral changes in zebrafish embryos can be applied for the investigation of pollutant release from MP. In fact, behavior has been shown to be highly sensitive to a wide range of (neurotoxic) pollutants and seems to be more susceptible than morphological parameters.

TU111

Influences of polystyrene microplastic particles on the toxicities of thiacloprid and methiocarb for the non-biting midge Chironomus riparius: mortality and biochemical responses

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Microplastics (MP) are of particular interest in ecotoxicology because they potentially interfere with organic substances like pesticides or pharmaceuticals, modulate their toxicity and in addition, may mechanically affect exposed organisms. Whereas in the past, most of the studies on MP toxicity have focused on the marine environment, the number of freshwater studies with respect to MP and dissolved contaminants has recently increased. The aim of the present study is to examine the influence of polystyrene (PS) particles (cryogenically milled granules, fractionated to < 50 μm) alone and in combination with the pesticides thiacloprid (TH) and methiocarb (MET) on the sediment dwelling larvae of the non-biting midge *Chironomus riparius.* \nIn the first experiment, 3rd - 4th instar larvae of *C. riparius* were exposed to PS particles (150,000 particles/L; MP), the insecticide TH (1μg/L) or the mixture of both (Mix) for 96 h. As endpoints, mortality rate and neurotoxicity (acetylcholinesterase inhibition) were investigated. The results showed a significantly increased mortality in all treatments containing TH (TH and Mix), compared to the negative control and the MP group. Furthermore, the Mix treatment showed a significantly lower mortality rate than the TH treatment, indicating that PS particles are able to reduce the lethality of TH. This effect has been shown in all five runs that have been performed. However, the underlying mechanism is not clear yet, and therefore, further investigations are necessary. The analyses of acetylcholinesterase activity showed no differences between the treatments. In a second experiment, we investigate the impact of different concentrations of the molluscicide MET alone and in combination with PS particles and TH on the health of *C. riparius*. The results will be presented on the poster. The study is part of the joint research project MiWa ('microplastics in the water cycle') funded by the German Federal Ministry of Education and Research (funding number: 02WRS1378).

TU112

Influence of differently functionalized polystyrene microplastics on the toxic effects of TiO2 NPs towards marine algae Chlorella sp.

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Increased utilization of titanium dioxide nanoparticles (TiO_2 NPs) for commercial as well as industrial purposes resulted in NPs accumulation in the marine system. Microplastics being an emerging secondary pollutant in the marine ecosystem have an impact on the toxic effects of TiO_2 NPs which has not been evaluated up to date. So it is important to assess the toxic effects of both these pollutants on marine environment.The present study examines the impact of differently functionalized microplastics on the toxic effects of P25 TiO_2 NPs to marine algae *Chlorella* sp. Tendency of NPs to undergo aggregation in artificial seawater was observed with increase in time. The median effective concentration for TiO_2 NPs was found to be 6.5 mg L^{-1} which indicates higher toxic effects of NPs toward algae. In contrast, microplastics irrespective of their difference in functionalization had minimal toxic effect of about 15% at their higher concentration tested. Plain and aminated polystyrene microplastics enhanced the TiO_2 NPs toxicity which was further validated with oxidative stress determination studies like reactive oxygen species and lipid peroxidation assays. Negatively charged carboxylated polystyrene microplastics decreased the TiO_2 NPs toxicity with possible heteroaggregation between TiO_2 NPs and microplastics in the system. The toxicity data obtained for the mixture was further corroborated with the mathematical model, Abbott.

Aquatic and Terrestrial Plant Ecology: Ecotoxicology, Risk Assessment and Modelling (P)

TU113

EVALUATION OF IN-VITRO PLANT METABOLISM AS A TOOL TO AID IDENTIFICATION OF METABOLITES FROM CROP METABOLISM STUDIES

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The identification of metabolites from crop studies can present a significant challenge analytically due to the complexity of the matrices involved and generally low concentrations observed. In the case of the confined rotational crop study (OECD 502) this is compounded by the fact that degradation may occur on the soil before uptake during the aging period and a general drive for lower application rates. In situations where if extensive metabolism is observed many metabolites can be generated, while the majority may be at or below the trigger value for identification (>50 ppb) many metabolites are found to be present at the characterisation requirement (10-50 ppb) level. According the study guideline characterisation does not necessarily include identification, however, if a residue definition is required it becomes necessary to identify these low level metabolites and it can be difficult to achieve accountability for the majority of the residue. While there have been many technological advances in analytical chemistry, particularly in the field of mass spectrometry and 2-dimensional chromatography which help with the identification of these metabolites the challenge of a complex matrix still remains. The identification of metabolites can become akin to looking for a needle in a haystack and clean-up procedures can be inefficient and costly. The use of *in-vitro* techniques may not produce metabolites in the same ratios that are observed in the whole plant, however, it is expected that the same metabolites are observed. An advantage of *in-vitro* incubation of plant cell cultures is that it allows the exposure rates of the agrochemical in question to be increased. This combined with near perfect growing conditions used for the incubation should produce metabolites in greater concentrations, in a cleaner matrix and in a shorter time than required to repeat the study (or part of) at a higher application rate. This presentation examines the viability and effectiveness of *in-vitro* plant metabolism techniques to provide a source of metabolites to aid metabolite identification in confined crop rotation studies.

TU114

Effects of graphene oxides on plant germination and growth

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Graphene oxide has a wide specific surface area and has excellent mechanical and thermal properties. Due to its superb material characteristics, GO has a high potential to be applied in various environmental fields. GO may enter the soils and groundwater as a result of such applications, but there is not much research on how GO may affect plants when they enter terrestrial ecosystems. To assess the impacts of GO on the germination and early growth of diverse plant species, we studied the response of radish, cucumber, lettuce, alfalfa, and perennial ryegrass to GO treatment. GO was treated to the seeds at the concentration of 0, 0.2, 0.4, 0.8, 1.6 mg ml^{-1} and we analyzed the germination rate and growth of plants. The germination rate of lettuce decreased as GO concentration increased. On the other hand, the effects of GO were not significant on the germination of other plants. The root and shoot growth of lettuce, radish, and alfalfa decreased with exposure to higher concentrations of GO. More specifically, the shoot and root length of lettuce decreased by 87% and 86%, respectively, at 1.6 mg ml^{-1}. Our results show that high concentrations of GO can inhibit the germination and early growth of plants. Therefore, we expect this study can help supplement the legal regulations for safe use of nanomaterials.

TU115

An assessment of levels of cyanide and heavy metals in mine soils and their effect on maize growth.

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We investigated the levels of cyanide and heavy metals in mine effluent and assessed their effects on maize growth. Soil samples were collected from a dump site of an operating mine and were tested for heavy metal residues and cyanide levels. Maize seeds were planted and allowed to grow in soil collected 200 m from the dumpsite for five weeks. Some maize seeds were planted and grown in control soil samples and irrigated with cyanide concentrations (0.05mg/L, 0.5 mg/L and 5 mg/L). The control maize seeds were planted and grown in control soil and were irrigated with tap water. The endpoint parameters for assessing growth were measurements of plant and leaf length. Oxidative stress was assessed by measuring the activities of catalase and superoxide dismutase. High concentrations of copper, zinc, cadmium and iron, recorded as; 401.59 mg/kg, 283, 85mg/kg, 22.855 mg/kg and 7353mg/kg respectively in soil from the mine dump site were observed. Cyanide concentrations of 1.25. mg/kg and 0.5 mg/kg from the mine dumpsite and soil collected 200 m from the dumpsite respectively were also recorded. Significant levels of heavy metal residues were observed in maize after five weeks of exposure to mine dump soil. Presence of cyanide and heavy metals had a negative effect on maize growth as growth was slowed when compared with the control plants. Antioxidant enzyme activity enzyme activity, both catalase and superoxide dismutase was significantly enhanced by exposure to contaminated mine soil. Our results show that contaminated mine soil impacts negatively on the health of plants grown in this soil.

TU116

INFLUENCE OF Cd2+ ON STRUCTURAL AND FUNCTIONAL PARAMETERS OF HALOPHYTE SUAEDA SALSA

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Halophytes comprise an ecological-physiological group of plants with a high potential for biological resistance to salinity. The resistance of halophytes to NaCl is associated with a variety of physiological and molecular mechanisms: limiting the flow of ions; salt extraction through glands; salt accumulation in vacuoles; participation of enzymes and low-molecular compounds in countering oxidative stress with antioxidant protection. Many of these mechanisms are also found in plants exhibiting high resistance to heavy metal ions (HM).The effect of Cd^{2+} ions on the structural and functional parameters of the halophyte *Suaeda salsa* was studied. The research task was to study the content of pigment, the intensity of lipid peroxidation (LPO) and the composition of membrane lipids. The objects of study were the leaves of *S. salsa* plants grown under laboratory conditions and wild-growing (Sw) plants collected under natural conditions in the basin of Lake Elton in southern Russia, and plants were grown in Green-House conditions (20–22 ° C, 1200 μmol / m^2 s^{-1}; containing Cd $(NO_3)_2$ 200 μM /L). The plants were divided into two groups: control (SK) and test samples (SCd).An assessment of the effect of NaCI and Cd^{2+} ions on plants of *S. salsa* did not reveal any visual signs indicating the toxicity of these two abiotic factors. The leaves of the SK and Sw plants are characterized by a high level of water content – 91–94 % of the fresh weight. The introduction of Cd^{2+} into the soil led to a decrease in water content in the roots of SCd. The highest content of ?hlorophylls and carotenoids was observed in plants grown on medium containing Cd^{2+}. Halophytes, which grew in natural conditions, were characterized by a lower content of photosynthetic pigments compared to the laboratory plants. In the leaves of SCd plants, a twofold increase in the intensity of POL was observed compared to SK. The total content of lipids in the leaves of the studied plants is 4.2–6.0 mg/g of fresh weight. In the composition of the main components forming the thylakoid membranes were observed rearrangements. The reaction of the pigment fund and lipids, responsible for the membrane structure, to the effects of Cd^{2+} ions in laboratory experiments was similar to the effect of soil salinity on plants under natural growing conditions.Thus, the mechanisms of resistance to NaCl and Cd^{2+} ions in halophytes are realized due to structural rearrangements of the membrane apparatus and activation of oxidative processes.

TU117

Predominance of gas exchange reduction over its recovery under heat and drought reflected in incomplete recovery of growth of spring oilseed rape even after exposure to elevated CO2 conditions

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In view of the current and growing impacts of climate change, the occurrence and severity of heat waves with extended drought periods undoubtedly will increase. Because of unique response of plants to combined treatments, recent studies highlight the importance of studying the impact of abiotic stresses on plants. So far, little it is known about the mitigating effect of elevated CO_2 on crop resistance to these stressors' combination. Hence, this study is aimed at the investigation of how an agronomical important crop spring oilseed rape (*Brassica napus* L.) will be able to cope with combined impact of heat wave (21/14 ºC *vs.* 33/26 ºC day/night) and drought with elevated CO_2 (400 *vs.* 800 μmol mol^{-1}) treatment and to what degree will manage their recovery after stress. The experiment was conducted in growth chambers under precisely controlled environment. Treatments were imposed at 13^{th} BBCH growth stage for 7 days and, after the cease of stress, plants were left for 7 days to recover under ambient climate conditions. The obtained results revealed that, at early vegetative stage, *B. napus* have rather high upper limit to temperature increase that allowed to perform them even better under +12 ºC heat wave compared to ambient climate conditions, in the presence of adequate water supply. However, the rate of recovery of gas exchange parameters of plants experienced combined heat and drought stress was significantly slower than its rate of reduction during the period of treatment that reflected in incoplete recovery of plant growth. Elevated CO_2 diminished the negative impact of drought stress, under heat wave conditions, on A_{sat} and shoot growth to a considerably extent. However, it had only a little effect on the recovery of A_{sat}, when additional CO_2 after stress treatment was also removed. After recovery period, both leaf area and shoot dry weight in drought-stressed spring oilseed rape's grown under heat wave conditions were significantly lower compared to control ones, regardless of CO_2 level.

TU118

Toxicity of MCPA herbicide on physiological laterations of lichen Ramalina fraxinea (L.) Ach.

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Phenoxy herbicides have been commercially available for over 60 years and are the most widely used family of herbicides worldwide. 4-Chloro-2-methylphenoxyacetic acid (MCPA) has been used in the United States, but has been the more widely used phenoxy herbicide in Europe, where it has been extensively used in forest management. The study aims to quantify the effects of increasing concentrations (0-100 mg/l) and the duration of exposure (0 and 24 h) of MCPA (phenoxy acid herbicide) on physiological status of the lichen *Ramalina fraxinea*. Results showed that MCPA exposure induced reduction in maximum capacity of PSII (F_V/F_M), chlorophyll *a* content along with chlorophyll degradation and adverse effects on cell membrane integrity. Measurements of physiological parameters indicated toxicity which was related with dose and time.

TU119

Measuring reproductive parameters in terrestrial plants - methods and challenges

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EFSA's Scientific Opinion addressing the state of the science on the risk assessment of plant protection products (PPP) for non-target terrestrial plants (2014) raises awareness of assessing for adverse effects on plant reproduction. Currently, effects of PPP on terrestrial plants are evaluated in seedling emergence and vegetative vigour studies completed in accordance with OECD and OCSPP guidelines. These tests involve pre- or post-emergence applications and subsequent maintenance of young plants up to 4 weeks under glasshouse conditions, during which time assessments are made of vegetative parameters, e.g. shoot height and biomass. In contrast, the measurement of reproductive endpoints requires maintenance of adult plants until seed set and harvest. As such, reproductive tests are significantly longer, more complex and challenging than the current guideline tests. The challenges surrounding the development of reproductive test methods include: Species selection: the diversity of reproductive strategies across plant families creates complexity for species selection. Specifically, consideration should be given to life-cycle (e.g. biennials may be less suitable than annuals), pollination requirements (e.g. insect pollination may be difficult under glasshouse conditions) and flowering/germination requirements (e.g. dormancy-breaking treatments, vernalisation). Maintenance of healthy plant growth: consideration needs to be given to provision of optimum nutrients over the test duration as well as practicalities (i.e. space requirements) of maintaining mature plants for long periods. Prolonging the cultivation period will significantly increase pest and disease pressure and may necessitate the use of control measures. Endpoint measurement: reproductive endpoints are numerous and diverse across species (e.g. flower numbers and morphology, fruit and seed numbers and weight, time to flowering and seed set, F1 seed viability) although the relative contribution of each parameter to the survival of the population is unclear. Selection of suitable endpoints requires consideration of the feasibility of measurement (e.g. seeds within fruits are difficult to count) and measurement of the same endpoint across all species may neither be possible nor practical. This presentation is intended to raise awareness of the challenges regarding reproduction testing in terrestrial plants.

TU120

A comparison between vegetative and reproductive endpoints for two non target plant studies with non-crop species in greenhouse and field

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Terrestrial plants are providing a broad spectrum of ecosystem services such as provisioning of e.g. food and natural medicines or the regulation of air quality (MEA 2005). Plants in the agricultural ecosystem can be divided into three basic groups: crop plant, target plants for herbicides (weeds) and non-target terrestrial plants (NTTP's). The main refuges for NTTP's in intensively used agricultural areas are field margins representing a semi-natural habitat. Since agriculture is the dominating land-use in the temperate zone protection of non-target plants is a regulatory aim. Using herbicides to reduce weed competition in agricultural areas can adversely affect non-target terrestrial plants growing at field margins. For a more realistic assessment of effects there are several suggestions to include also non-crop species in the testing scheme from the list provided in OECD guidelines (OECD 208 and 227) and to assess the life-cycle with flowering and seed production. The general objective of this work was to assess if a greenhouse and a field study with a similar experimental design results in a comparable outcome for vegetative and reproductive endpoints. The experimental design used in booth studies consists of one control and four herbicide application rates with the same herbicide product. In the greenhouse 6 replicates per tested rate were used while in the field 8 replicates were used to account for the higher variability in the field. Furthermore in the greenhouse species were grown individually while the field study consisted of plant communities established from seeds. The four rates included the field rate down to a 3% drift rate. Assessed were biomass and plant height at the vegetative and generative growing phase of the plants. The number of flowers and seeds and germination of the harvested seeds were assessed for selected species to evaluate differences in flowering and germination. Additionally, in the field study effects on plant community were assessed with multivariate analysis. MDDs were calculated for individual endpoints to see what the effect levels are that can be detected. The presented results will focus on the recovery of the vegetative endpoints biomass and plant height over time and on the reduction of seed production in both studies and the reduction plant frequency in the field study References: Millennium Ecosystem Assessment (MEA) (2005) Ecosystems and human well-being: synthesis. Island, Washington, DC

TU121

Predicting plant uptake of ionic chemicals: Redefining the dominant processes

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Several tools exist to predict uptake of neutral chemicals into plants. For most of them, partition coefficients (Kow and Kaw) are highly relevant input parameters, and dominant processes are sorption and volatilisation. A few of the prediction tools are also considering chemical speciation (based on log D, pKa, pH) and are therefore, supposedly, applicable to ionizable chemicals (like most pharmaceuticals and pesticides). However, successful applications are rarely reported. We suspect that the reason for this lack is that polar, ionizable substances undergo, to a much higher degree, processes which are not well described. The dominant processes for ionic species are no longer sorption and evaporation, but rather: i) transformation / metabolism ii) ionization / ion trap / electrical interactions So far, both i) and ii) can hardly be predicted. A physically-based complex dynamic plant uptake model has been developed describing the uptake of ionizable chemicals down to a cellular level. Such a model holds the potential to describe the uptake and distribution in plants in a site- and chemical specific way. But it comes with the price of very many input parameters, i.e. complexity rather than parsimony. Sensitivity analyses reveal that the most sensitive parameters are not the usual suspects as hydrophobicity, volatility, growth rate and transpiration rate, but rather pH inside sap streams and cells, cell permeability to ions and descriptors of protein adsorption. Furthermore, also reaction and transformation rates are highly sensitive, in particular for polar and ionic compounds. These new sensitive parameters are not well described, leading to wide uncertainty ("semi-chaotic" behavior) and thus bad prediction power of the model - despite high effort. The same wide range of results can also be seen when comparing experimental results from literature. Work is currently being done to reduce the input parameter space and to minimize the uncertainty. The model is being improved and tested against various datasets. The present version of the model can be downloaded for free in an excel implementation from https://homepage.env.dtu.dk/stt/2017Release_Plant_Model/index.htm

TU122

Influence of drift interception by canopies on herbicide effects on non-target terrestrial plants

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This field study was designed to determine the distance downwind from a treated area at which the biomass of non-target terrestrial plants (NTTP) is no longer significantly affected by applications of an herbicide formulation ("no observed effect distance"). In addition, influence of downwind vegetation on the effects of the herbicide on NTTPs was assessed. Navy bean and lettuce plants have been shown to be sensitive to the herbicide under investigation in standard greenhouse studies. Potted plants of each species (n = 25 for each species) were placed downwind of an application area on either bare ground (worst-case exposure) or in the off-crop edge vegetation (typical exposure). The field portion of the study was done in October 2018 at an agricultural site in Stilwell, KS; USA in Johnson County using a ground boom sprayer and coarse spray nozzles. Potted plants were placed in staggered rows at locations approximately 1.5, 3, 6, 15 and 30 m (5, 10, 20, 50, and 100 ft) downwind from the edge of the application area. For each exposure scenario, there were four spray swaths perpendicular to the off-field transects. Following spray application, the potted plants were transported to an indoor greenhouse area and maintained for an additional 21 or 28 days. Biological measurements were monitored per standard vegetative vigour guideline (EPA). Clear differences were observable in the effects for both test species between the test plants placed in edge vegetation and those plants placed on bare ground. Results suggest that typical exposure of NTTPs in vegetated off-field areas by herbicide drift leads to less pronounced effects than predicted by exposure on bare ground. Furthermore, this study supports the conclusion that standard greenhouse studies may seriously over-predict the effects of herbicide drift onto non-target plants.

TU123

A graphical user interface for applying the plant community model IBC-grass in ecological risk assessments

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Ecological risk assessments aim at estimating the potential risk for the environment via a tiered approach. If standard greenhouse tests on individual-level fail, higher tier options can be conducted to more accurately evaluate potential undesirable effects on populations or communities. In the EFSA opinion on potential higher tier options for non-target terrestrial plants, modeling studies are mentioned. However, ecological models are often very complex and good modeling knowledge is necessary in order to use such models. In addition, regulators are often not modelers and a lack of modeling experience may lead to hesitation by regulators to use model predictions in their decision making. Thus, there is an increasing need in facilitating the use of ecological models for risk assessors and regulators. In the last few years, we refined the plant community model IBC-grass to be a useful tool for extrapolating individual-level effects based on standard greenhouse experiments to community-level. To facilitate the application of the model in risk assessments of non-target terrestrial plants, we developed a graphical user interface (GUI), which can be used also by people not familiar with ecological models. The software is available for free and comes with the user manual and the good modeling practice (GMP) document. The GMP document gives the user an overview of the model development, processes included in the model, outcomes of sensitivity analyses and validation of the model. It should help regulators to understand the model and to decide whether to use the model for their decision making. In this poster presentation we will introduce the GUI and show an exemplary application.

TU124

Simulation model to study percolation toxicity of pesticides, isolated and in mixtures, in Raphidocelis subcapitata

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Pesticides have been recognized worldwide for their importance in the global loss of biodiversity in aquatic environments and the functioning of ecosystems. In the aquatic environment, algae play an important role as the basis of the food chain and effects on their communities can impact higher trophic levels by altering the functioning of the ecosystem as a whole. However, even with these environmental impacts, Brazil is the largest consumer of pesticides in the world, which justifies the need to study the toxicity of these compounds in the biological community. Another crescent preoccupation is about the global warming and impact in ecosystems, once, the temperature can alter the distribution environmental contaminants. Considering that, this research was to assess the effects of pesticides Kraft®and Score®(isolated and simple mixture) largest used in Brazil, on *Raphidocelis subcapitata*. The experiments were carried out with four treatments (Kraft, Score, Kraft+Score and Control), in simulators filled with 0.1 $m^3$of soil, which received an application of 4.8µL Kraft and 1.3µL of Score, diluted in 1L, being reapplied every seven days, following manufacturer's application instructions, and performed at two temperatures of 23ºC and 33ºC. On the experimental units, 6 rains (twice a week) were simulated, in which the percolated material of the rains was used to carry out the tests with the algae, following the ABNT NBR 12648, and analyzed population growth rate by flow cytometry. Data were analyzed using Analysis of Variance (ANOVA), followed by Dunnett. Analyzing the physical and chemical variables, it was verified that they have remained within the established for the test species. For nutrients, total nitrogen, total phosphorus, nitrate, ammonia, total phosphorus dissolved were analyzed to characterize percolate, and even with high values, between 1000 - 10000, were almost the same for both temperatures tested. The results showed high Kraft toxicity, in both temperature tested. In 23ºC, were significant difference of control in two experiments (2 rains) realized, in the first application the pesticide mixture affected algae growth and in third, all the treatments were different from the control. To 33ºC, the growth of *R. subcapitata*was affected by Kraft in the first and in the second application, even as in the mixture. Which indicate that the temperature interferes in the pesticides toxic effect under non-target organisms.

TU125

Evaluation of the effect of caffeine on the green microalgae Monoraphidium sp. (chlorophyta)

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In this study the effects in: growth, chlorophyll concentration, carotenoid concentration, phenol concentration and lipoperoxidation degree (Tbars) of the green microalgae *Monoraphidium* sp. caused by exposure to caffeine were determined, because at present, caffeine is considered an emerging pollutant. Static bioassays were performed with a duration of 72 hours. The microalgae were exposed to 5 caffeine concentrations (0.025, 0.05, 0.5 5, 50 and 100 mg L^{-1}) in triplicate to determine the EC_{50} (effective concentration 50). Subsequently, a sublethal bioassay was carried out where the microalgae were exposed to concentrations of 0.025, 0.05, 0.5, 50 mg L^{-1} plus a control group for 8 days. At the end of the exposure period, the population growth, the concentrations of chlorophyll *a* and *b*, carotenoids, phenols and degree of lipoperoxidation were evaluated. The results obtained showed that there were significant differences between the responses of the control group and the microalgae exposed to caffeine ($p < 0.05$). The lowest levels of total chlorophylls were obtained in the concentration of 0.025 mg L^{-1}, but an increase in the concentration of chlorophylls (a and b) in the concentrations of 0.5, 5 and 50 mg L^{-1} was observed. A higher production of carotenoids compared to the control group was detected in all the cases. An increase in the production of phenols was recorded in the tests with caffeine concentrations of 0.05, 0.5, 5 and 50 mg L^{-1}. The average levels of Tbars recorded in the bioassays with the concentrations of 0.05, 0.5, 5 and 50 mg L^{-1}., were similar to those recorded in the control group. Caffeine did not affect the growth of microalgae at the concentrations tested in this study.

TU126

Comparative sensitivity of Lemna gibba and Lemna minor to 3,5-dichlorophenol

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Assessment of environmental risk requires toxicity test on aquatic plant to determine potential impact of contaminates using primary producers. Many research have proved the difference in sensitivity across phylogenetic groups of plant. In the present study, an ecological impact of 3,5-dichlorophenol (DCP) was assessed using the aquatic floating vascular plants, *Lemna gibba* and *Lemna minor*. The sensitivity of several ecologically relevant parameters namely presence of DCP in test media, primary measurement variable, number of fronds and one other measurement variable, dry weight were compared after 7 day exposure to DCP. Cultures were assessed visually for plant development. Yield and growth rate inhibition for frond and dry weight were calculated at the end of experiment. Results indicate concentration of 3,5-dichlorophenol was maintained >80% in test medium throughout the experiment. Doubling time of frond number of *L. gibba* and *L. minor* in the control was 1.93 (corresponding to 12.5 times increase in seven days) and 1.58 (corresponding to 21.39 times increase in seven days) days, and an average specific growth rate of control was 0.36/d and 0.44/d, respectively. *L. gibba*, growth rate and yield inhibition EC_{50} for frond was 10.46 and 6.92 mg/L, respectively while for dry weight was 7.27 and 5.44 mg/L, respectively. Based on results of the statistical analysis, the NOEC and LOEC for growth rate and yield of frond were 2.7 and 4.4 mg/L, respectively and for growth rate and yield of dry weight were 4.4 and 7.0 mg/L, respectively. *L minor*, growth rate and yield inhibition EC_{50} for frond was 2.75 and 1.61 mg/L, respectively while for dry weight was 1.54 and 1.16 mg/L, respectively. Based on results of the statistical analysis, the NOEC and LOEC for growth rate of frond were 0.7 and 1.5 mg/L and yield of frond were 0.3 and 0.7 mg/L, respectively. The NOEC and LOEC for growth rate and yield of dry weight were 0.3 and 0.7 mg/L, respectively. Finding postulates that *L. minor* ismore sensitive than *L. gibba* for DCP. Through testing the phytotoxicity of DCP to *L. gibba* and *L. minor*, it is evident that there are considerable differences in sensitivity among species and that the selection of species is necessary to provide an acceptable margin of safety in evaluating the hazard untaken by the chemicals to the aquatic environment.

TU127

Impacts of contaminants in freshwater macrophyte species

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In the last decades, the use of pesticides has been intensified mainly in European Mediterranean regions, and in some cases exceeding the limits of regular legislations established by the European Union. The intensive use of the chemicals on agricultural fields surrounding aquatic systems may comport consequences to the ecosystems and communities. Primextra® Gold TZ is one of the most used herbicides in corn crop fields of the Mondego valley, according to the information from agricultural cooperatives. The main active ingredients of the herbicide are s-metolachlor and terbuthylazine. Moreover, copper based formulations, such as copper sulphate, are also quite used in the agriculture practice. Thus, this work pretended to evaluate the effects of these chemicals (s-metolachlor, terbuthylazine and copper sulphate) in the non-target species *Lemna minor* and *L. gibba*, two freshwater macrophytes, reported as a standard species in ecotoxicological bioassays. In a first step, the organisms were exposed to the contaminants under laboratory conditions, after which the biochemical analysis to determine changes on the nutritional value was performed through the determination of fatty acids, polysaccharides and free sugars profiles. The results showed *L. gibba* ($LC_{50Copper}$ =0.459 mg/L; $LC_{50S\text{-}Metolachlor}$ =0.087 mg/L) was more tolerant than *L. minor* ($LC_{50Copper}$= 0.207 mg/L; $LC_{50S\text{-}Metolachlor}$ =0.043 mg/L) when exposed to copper and s-metolachlor. However, the opposite trend was observed when macrophytes were exposed to terbuthylazine, with *L. minor* (LC_{50TBZ} =0.0934 mg/L) demonstrating higher sensitive to this chemical action than *L. gibba* (LC_{50TBZ} =0.0439 mg/L). Furthermore, the composition on lipids and carbohydrates was also changed with the exposure to all compounds for both species. Therefore, biochemical biomarkers are important tools and endpoints in ecotoxicological studies and may be used as early-warning indicators of the presence of contaminants at the ecosystems and on the determination of potential effects in their communities. This study was supported by Fundação para a Ciência e a Tecnologia (FCT) through the strategic projects UID/AMB/50017/2013 granted to CESAM and UID/MAR/04292/2013 granted to MARE. FCT funded A.F. Mesquita, A. M. M. Gonçalves, C. Nunes and A. P. Cuco (SFRH/BD/139831/2018; SFRH/BPD/97210/2013; SFRH/BPD/100627/2014 and DPA 17-781). The research was partially supported through the project Ref. POCI-01-0145-FEDER-022127.

TU128

Mode of action and adverse effects of gamma radiation in the aquatic macrophyte Lemna minor

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Ionizing radiation has been reported to cause adverse effects in different primary producers including both autotrophs and heterotrophs. Upon exposure, high dose rates of ionizing radiation can reduce reproduction and growth, cause damage to DNA and other biomolecules. However, the physiological responses and toxicity mechanisms occurring at low doses and dose rates are still poorly understood in aquatic plants. This study aimed at characterizing the biological effects of ionizing (gamma) radiation from a cobolt-60 (^{60}Co) irradiation source using a combination of Mode of Action (MoA) characterization and determination of adverse (apical) effects in duckweed (*Lemna minor*). Chronic toxicity was assessed as inhibition of growth after 7 days exposure to 1-70 mGy/h according to the OECD test guideline 221 (OECD,2006). The MoA of gamma radiation was determined as oxidative stress (ROS and lipid peroxide formation) and DNA damage in combination with physiological responses such as oxidative phosphorylation (OXPHOS) and Ca^{2+} release, photosystem II (PS II) activity, CO_2 assimilation, antioxidant responses (total GSH), and pigment changes (Chlorophyll *a*, Chlorophyll *b* and carotenoids). The results indicated that changes to tGSH, pigments, CO_2 uptake, Ca^{2+} release and DNA occurred at dose rates between 1 and 14 mGy/h, whereas ROS formation and lipid peroxidation (LPO) together with decrease in OXPHOS and growth parameters were only observed at dose rates of 24 mGy/h and higher. An Adverse Outcome Pathway (AOP) network was developed to portray causal relationships between gamma radiation induced physiological responses and adverse outcomes in *L. minor*.

TU129

Simple CA and IA models underestimate the phytotoxicity of micropollutants caffeine and nonylphenol

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Given the prevalence of micropollutants in aquatic systems, the assessment of mixture effects is vital to a complete understanding of the effects of chemicals in the environment. Caffeine and nonylphenol are among the most commonly detected micropollutants in freshwater systems but come from different chemical classes and differ greatly in their individual potencies. Two major models dominate the assessment of mixture toxicity: concentration addition (CA), which is typically applied to chemicals with the same mode of action, and independent action (IA), which is applied to chemicals with different modes of action. Single chemical tests and an equitoxic binary mixture test were performed with the aquatic macrophyte *Lemna minor* to assess the potential for interaction between these commonly co-occurring chemicals. Seven day exposures were conducted with endpoints including frond number, frond area, fresh weight and chlorophyll content. Concentration-response curves from the single chemical tests were used to select equitoxic mixture ratios for the mixture test, and the results were then compared to the predicted mixture effect based on CA and IA models. In the single chemical tests, caffeine did not have high phytotoxicity on its own, indicating effects at environmental concentrations are unlikely. The toxicity of nonylphenol was greater but limited by nonylphenol's water solubility and associated with higher variability. Significant growth inhibition, tissue necrosis and chlorophyll alterations were detected at only one order of magnitude from the highest reported environmental concentrations, suggesting environmental effects from nonylphenol exposure are possible. IA underestimated the toxicity of the caffeine-nonylphenol mixture by at least a factor of two at all effect levels. CA also underestimated the mixture toxicity, but the model deviation ratio was under two for the tested effect levels and wide confidence intervals overlapped with the mixture regression obtained from experimental data. Based on these results, synergistic interaction between caffeine and nonylphenol is possible. However, CA cannot be ruled out as an effective model if the uncertainty could be reduced. The ambiguity of the results indicates the difficulty of choosing suitable mixture models in non-ideal cases.

TU130

Modelling growth of Lemna exposed to Metsulfuron-methyl using a dynamic energy budget approach

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The exposure of pesticides are dynamic and variable in time. However due to the complexity of the exposure profile, it is not possible to study every exposure in a laboratory experiment. To allow extrapolation to any predicted exposure profile, we model effects on growth using a simplified dynamic energy budget model (based on DEBkiss). This model is a mechanistic approach relying on mass and energy balance, which is suitable for the sublethal toxicity endpoint growth. We compare our model's performance with another *Lemna* model, developed by Schmitt et al. 2013. As a case study, we consider the sulfonyl-urea herbicide Metsulfuron-methyl (MSM).

TU131

Old herbicide in new light: filling the data gap of mecoprop-p for dicotyledonous macrophytes

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The fate and concentrations of the auxin phytohormon herbicide mecoprop-p (MCPP-P) in the aquatic environment is well documented but little toxicity data of dicotyledonous aquatic macrophytes have been published so far over the last decades. To fill up this data gap, the German Environment Agency (UBA) conducted a microcosm study in order to test the sensitivity of 10 different macrophytes (mainly dicotyledonous species). Ten macrophyte species (*Callitriche palustris, Ceratophyllum demersum, Hottonia palustris, Hydrocotyle leucocephala, Hydrophila polysperma, Ludwigia repens, Myriophyllum spicatum, Nymphoides peltata, Ranunculus aquatilis*, and *Veronica beccabunga*) were exposed to MCPP-P in 7 concentrations ranging from 8 μg/L to 512 μg/L for 21 days in each one microcosm. Two further microcosms served as controls. For each species, ten individuals were planted separately in plant pots, which were filled up with 2 layers of sand (bottom and top) and a middle layer of a mixture of commercial pond soil and sand. Plant preparation and endpoints were adapted to each species but in general fresh weight, dry weight, length of main shoot, and number of leaves/whorls were measured at the start and at the end of the experiment. Eight species proofed to be sensitive to MCPP-P. For 5 species it was possible to generate EC_{50} values ranging from 47 to 444 μg/L MCPP-P with *M. spicatum* being one of the most senstive species. On base on data of this study, it was possible to construct a species sensitivity distribution (SSD) model combining other published data. The results will be critically discussed. Our test confirms the reliability of the use of *M. spicatum* as representing dicotyledonous species, as it proved to be one of the most sensitive species in our study for the tested auxin herbicide. Regarding the new low RAC concentration of MCPP-P, it has to be highlighted that uses like in bitumous roofing felts are not in the focus of any regulation so far. This application, however, can lead to high run-off concentrations entering surface waters exceeding the RAC values.

TU132

Macrophyte Toxicity Testing: Influence of Growth Form on Sensitivity

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The importance of macrophytes in maintaining biodiversity in the ecosystem cannot be understated. They provide food, habitat and affect the physical and chemical properties of aquatic systems. The current guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters involves performing toxicity test on macrophytes, green algae, additional non-green algae, and on *Lemna* species. In case the herbicide simulates a plant growth hormone or if *Lemna* species is not sensitive to the herbicide, then testing with *Myriophyllum spicatum* is recommended. Testing on *Glyceria maxima* is also recommended in case the action of the herbicide is primarily on monocots. Furthermore, additional aquatic plants can be tested in case the uncertainties in the outcome of the risk assessment were high in order to refine the risk assessment process. In the current study, toxicity tests were performed using six rooted aquatic macrophytes. These were namely *Myriophyllum aquaticum, Hygrophila polysperma, Limnophila sessiliflora, Callitriche pallustris, Nasturtium officinale and Heteranthera zosterifolia.* These aquatic plants can grow emersed for example during periods of drought and for example submersed during flooding. Also the different forms can be built in the same habitat. Based on these findings, the different growth forms of these species were tested. The aquatic plant species were obtained from commercial sources and adapted in both their emersed and submersed states prior to the test. 3,5-Dichlorophenol (DCP) was used as the test substance and the EC_{50} of the mean growth rates and yields were determined and compared for each species. The results obtained showed that, the different growth forms of the plants had an influence on the sensitivity of the plant species to the test substance. Preliminary results also indicated that, most aquatic plant species which were adapted to the emersed state demonstrated lower sensitivity to 3,5-DCP compared to those adapted to the submersed state.

TU133

A draft OECD Test Guideline for the emergent macrophyte, Glyceria maxima, in a water-sediment system: results of a ring-test with Imazapyr

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Data requirements introduced under EU Directive 1107/2009 stipulate that tests with aquatic plant species other than *Lemna* may be required for plant protection products which show selectively higher toxicity to either dicotyledonous or monocotyledonous plant species in terrestrial plant tests. In these cases, the recommended dicot and monocot species are *Myriophyllum* and *Glyceria*, respectively. OECD Test Guideline 239 for testing *Myriophyllum spicatum* in a water-sediment system was adapted to facilitate growth of the emergent, reed grass, *Glyceria maxima*, and ring-tested in 13 laboratories during 2016 and 2017 with the herbicide, isoproturon. Results from this ring-test were used to adapt the test protocol in terms of plant propagation recommendations, test system specification, test duration, assessment parameters and draft validity criteria. This revised protocol was used in a second ring-test with the herbicide, imazapyr in 11 laboratories during the Summer/Autumn of 2018. In addition, this work was submitted to OECD for consideration as a new Test Guideline in November 2018. This presentation will summarise results from the imazapyr ring-test and discuss progress towards delivery of an OECD Test Guideline.

TU134
Regime shifts in freshwater ecosystems exposed to multiple stressors by increasing temperature, fertilizers and pesticides (CLIMSHIFT)
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This poster will present the aims and first results of our French-German collaborative project CLIMSHIFT. Shallow freshwater habitats provide vital ecosystem functions but are threatened by multiple stressors. While a response to global climate change might be gradual, abrupt changes are possible when critical thresholds by additional effects of local stressors are exceeded. The difficulty in analysing effects of multiple stressors is to account for complexity, as stressors may act additive, synergistic or antagonistic. CLIMSHIFT aims for a mechanistic understanding of stressor interactions acting on shallow aquatic systems, which are especially vulnerable to climate warming and agricultural run-off due to their high surface to water ratios, large riparian interface and groundwater connectivity. Complex interactions between different functional groups of benthic and pelagic primary producers and associated consumers result in alternative stable regimes. Multiple stressors may trigger non-linear shifts between those regimes, with far-reaching effects on crucial ecosystem processes and functions. Our main hypothesis is that increased temperature will enhance negative effects of agricultural run-off, containing nitrates, pesticides and copper. Submerged plants, periphyton and phytoplankton as primary producers will be combined with the second trophic level, consumers, composed of the snail Lymnaea, consuming periphyton and plants, and benthic and pelagic phytoplankton filter-feeders, Dreissena and Daphnia. We will apply exposure scenarios at two different spatial scales to understand effects at the individual, community and ecosystem level. Investigations in microcosms at laboratory scale will be upscaled to larger, outdoor mesocosms. We will use an integrative dynamical model approach to simulate potential outcomes and critical thresholds, and predict stressor interactions. We expect that combined stressors will lead to sudden shifts in community structure in highly coupled systems. Macrophytes are expected to be replaced by phytoplankton or benthic algae, with major consequences for important ecosystem functions. Common ecotoxicological stress indicators such as growth and biomarkers of the different organisms will be combined with functional community/ecosystem response factors. The outcome of our project will support de definition of “safe operating spaces” for a sustainable agriculture and management of shallow aquatic systems in a changing world.

TU135
Phytotoxic effects of the hydrolate of three aromatic plants with pesticidal properties
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The TECHNOBIOCROP Project (Biopesticide development by chemical and biotechnological tools) focuses on the identification of biopesticides that could be a good alternative to pesticides of synthetic origin because of their effectiveness but also because their effect could be less harmful to the environment. In the process of extracting essential oils by vapour pressure extraction, there are other products, such as the hydrolates, that have been usually considered as by-products and are less studied. These aqueous extracts have water-soluble volatile compounds and recently showed numerous bioactive ingredients. In this study, we analyzed the phytotoxic effect of the hydrolates from three aromatic plants that have shown interesting properties as plant/fungal/agriwaste-based crop protectants: *Artemisia absinthium, Lavandula luisieri and Satureja montana*.The phytotoxicity of these hydrolates was studied through monitoring their impact on the root growth of onions (*Allium cepa* L.). The three hydrolates were able to inhibit the root growth of *A.cepa* at very low concentrations, within a range of LC_{50} values between 0.022 to 0.054 v/v. The phytotoxicity of the hydrolates from the highest to the lowest was: *Lavandula luisieri*> *Artemisia absinthium*> *Satureja montana.* This study allows continuing with the research of the effects of new biopesticides from hydrolate fractions on the environment and especially on non-target organisms, such as plants, in order to follow the regulations that will allow their commercialization. The authors thank the financial support of MINECO-FEDER (CTQ2015-64049-C3-2-R) and Gobierno de Aragón-FSE (GATHERS E39_17R).

TU136
SETAC Plants Interest Group
H. Krueger, EAG Laboratories
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New Insights into Chemical Exposures over Multiple Spatial and Temporal Scales (P)

TU137
Spatial and temporal distribution of 41 pharmaceuticals in European rivers
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Despite significant research over the last two decades, the spatial and temporal resolution of active pharmaceutical ingredients (APIs) occurring in wastewater, their removal in water treatment and fate in the aquatic environment remains unclear. Here we conduct targeted monitoring of 41 APIs in seven rivers in: Odense, Denmark (n=1), Dulman, Germany (n=1), Santiago de Compostela, Spain (n=1) and York, Barnsley and Durham in the United Kingdom (n=4) over one year. In addition to river water, influent and effluent samples were collected from wastewater treatment plants (WWTPs) discharging to all rivers except in the York catchment. Wastewater samples were also collected quarterly from sewers discharging from hospitals and residential areas in Odense, Santiago de Compostela and Durham. All samples (n=337) were 10mL of water collected from a total of 36 sampling locations on a monthly basis between March 2017 and February 2018. Samples were filtered with a glass microfiber syringe filter (0.7 μm pore size) then frozen on dry ice in the field before analysis at the University of York, York, UK. Shipment occurred overnight on dry ice and samples were stored at -20?C until analysis. Quantification was achieved using an in-house validated method for HPLC-MS/MS. Significant differences in the composition of wastewater by therapeutic class of API were observed in both waste- and river water between all the studied waste and rivers systems. No clear difference was observed in the composition of wastewater by therapeutic class between sewers originating from hospitals and residential areas respectively. Furthermore, these compositions and concentrations remained relatively unchanged between sewers and WWTP influent and were consistently dominated by the anti-hyperglycaemic drug metformin and analgesic paracetamol. Overall mean API removal efficiencies at WWTPs ranged from 83% (Dulman, Germany) to 35% (Barnsley, UK). Clear spatial and temporal patterns emerged in the monitored rivers, largely driven by WWTP discharge and variation in flow/ seasonal changes in API use respectively. Highest sum concentrations were observed in the River Sar (Santiago de Compostela) with a mean 17.6μg/L total concentration while the lowest was observed in the York catchment (Rivers Ouse and Foss) with a 2.1μg/L total concentration. These data indicate that both temporal and spatial considerations are needed to develop a complete picture of API fate in the built and aquatic environments.

TU138
Simulating behaviour of petroleum products during sewage water treatment
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Petroleum products are complex mixtures of chemical compounds. The physical-chemical properies of these compounds show a wide variety and as a result, will behave differently, but predictable, upon release to the environment. An approach to assess the risk of petroleum products is the hydrocarbon block method (HBM). With the HBM and the multimedia model SimpleTreat, distribution and elimination of petroleum products can be predicted in aerobic wastewater treatment plants (WWTP). A major feature of this model is that it only requires a few basic properties of the chemical to calculate emissions to air, surface water and in produced sludge (sometimes applied to agricultural soil). Furthermore, in recognition of the complexity of petroleum products, more advanced treatment processes are constructed in WWTP. So, it would be of value to upgrade SimpleTreat with more advanced treatment processes, such as dissolved air flotation (DAF). DAF is a process for removing suspended particles from liquid by bringing the particles to the surface of the liquid. When this process is added to SimpleTreat, it is important to understand how including DAF affects the concentration predictions of petroleum products. The aim of this study is to adapt SimpleTreat for simulating behaviour of petroleum products during sewage treatment. The intended use of this re-parametrized model is focussed on risk assessment of petroleum products. Modelling was performed using SimpleTreat 4.0 and the CONCAWE library. In SimpleTreat 4.0 a simulation of DAF was

included. Furthermore, chemical fate in WWTP of the library compounds was modelled for SimpleTreat 3.1, SimpleTreat 4.0. The latter with and without simulation of DAF. Comparison of model outcome o will give insight in the effect of changes in SimpleTreat. It appeared that the upgrade of SimpleTreat to 4.0 has an effect on the fate in WWTP. The most influencing parameter for these calculations seemed to be the hydraulic retention time. However, the outcome of these calculations still needs to be validated. The influence of including the DAF process in the model will also be shown. Our study explains the added value of using an up-to date multimedia model to get a more reliable insight in the risk of petroleum products.

TU139

Quantification of 9 bisphenol analogues in total blood, plasma and urine samples from a cohort of workers through a DLLME extraction followed by GC-MS determination

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Endocrine disruptors (ED) are a wide group of chemicals that can interfere with the normal function of the endocrine system, causing harmful effects on human health. Among them, there is the group of bisphenols (BPs), synthetic chemicals widely-used for the manufacture of polycarbonate plastics and epoxy resins. Due to their hardness, transparency and high strength over a wide range of temperatures, BPs are extensively used by the industry in food contact materials, such as coatings and linings of drink cans and reusable bottles, medical equipment and thermal paper. Due to the widespread use of these chemicals, exposure cannot be avoided; therefore, an accurate exposure assessment is required for the protection of human health. The aim of this study was to assess the exposure to 9 bisphenols (A, AF, F, B, S, AP, P, S and Z) in total blood, plasma and urine samples from a cohort of workers at a hazardous waste incinerator located in Tarragona (Catalonia, Spain). Free and conjugated BPs were determined through a dispersive liquid-liquid microextraction (DLLME) which combines extraction, derivatization and concentration in only one step. This was followed by a gas chromatography coupled to mass spectrometry. Only BPA was found in all biological matrices. In urine, free BPA was detected in 5 out of 25 samples (20%) and 8 samples presented values below the limit of quantification (< 0.1 μg/L). In turn, total BPA (conjugated + free) was found in 13 samples in concentrations ranging from 0.1 to 2.820 μg/L. For the total blood samples, free BPA was detected in 11 samples (0.27 – 1.65 μg/L) while total BPA was detected in 13 samples (0.04 – 1.52 μg/L). BPB was also detected in 3 blood samples in higher concentrations than BPA (2.51, 2.81 and 2.53 μg/L). Total BPB was only detected in a single sample of blood (2.69 μg/L), while BPA was detected in 3 out of 5 plasma samples, in concentrations ranging from 1.06 to 13.4 μg/L. BP concentrations were correlated with available data regarding levels of some organic compounds and heavy metals, previously determined in the same samples of plasma, urine and blood.

TU140

Bisphenol A: Reassessment of sources and emission pathways for FlowEQ-model assisted estimation of surface water concentrations

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*Flow*EQ, a hybrid fate and transport model that combines the flow network of the MORE/MONERIS model with a Level III fugacity-based model, was previously used for the assessment of Bisphenol A (BPA) in the environment. Best estimate emissions revealed total loadings of approximately 9 t/a into surface waters in Germany. As *Flow*EQ is parametrized to test the impacts of various pathways, the model results indicated that emissions from municipal wastewater dominate in virtually all geographic areas and have a much larger contribution to surface water concentrations than industrial and manufacturing emissions. While specific sources could not be identified, a follow-up study was performed considering updated market segment ratios and applied BPA release rates into water for interior and exterior use scenarios of polycarbonate (PC) and epoxy resin (EpR) based articles. *Flow*EQ results based on existing migration data suggested that PC and EpR based articles contribute to only about 1 % of total BPA loadings to surface water during their use phase and disposal. In a recent, extended campaign, measurements for > 20 wastewater treatment plants (WWTPs, influent/effluent, sewage sludge) and > 50 leachate samples from > 20 landfills were performed. In addition, >> 100 articles covering 18 article classes were analyzed for their BPA content. Highest influent BPA concentrations (up to 905 μg L^{-1}) were found in WWTPs affected *i.a.* by discharges from paper processing (*e.g.*, recycling), simultaneously leading to highest concentrations in sewage sludge (up to 200 mg kg^{-1} d.w.). Most WWTPs showed BPA removal rates in the range of 80-100 %, significantly lower rates were observed in four WWTPs. The reason for such low performance is not clear. BPA levels in landfill leachates were largely different (up to 52,000 μg L^{-1} prior treatment) and correlated with the starting date of landfilling for still operating sites. Of the >> 100 article samples analyzed, highest BPA concentrations were detected in various articles made of recycled PVC (up to 1,691,700 μg kg^{-1}), in recycled PVC granules (up to 204,800 μg kg^{-1}) and products made from recycled paper (up to 48,400 μg kg^{-1}). *Flow*EQ modeling using refined input parameters is able to attribute a significant part of the annual BPA emissions and the resultant concentrations in surface water to specific sources. It may thus serve as an important tool to model alternative emission scenarios and explore how concentrations change as usage patterns and treatment technologies change.

TU141

Different Sources of PCBs Generation in Wastes and their Presence at Dilijan Landfills (Republic of Armenia)

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Polychlorinated biphenyls (PCBs) are a group of organic compounds with a wide distribution, high toxicity, persistence and ability to long-distance migration. PCBs differ by unique properties: temperature stability; high boiling point; non-combustibility; resistance to chemical and physical influences; high dielectric constants. Such features allow PCBs versatile application as heat transfer fluids in heat exchangers, dielectrics in transformers and capacitors, fluids for hydraulic systems, plasticizers in lacquers, plastic masses, printing inks, copy paper, lubricants, insulating materials for cables and wires, fungicides for protection of building structures and wood, fillers for pesticides, additives to household chemicals, substitutes for wax, resins and rubber for impregnating fabrics. Sources of PCBs generation and spread in the environment are diverse, as they are used not only in the energy sector. Landfills of Dilijan district, where wastes are disposed without prior separation, were investigated. Determination was carried out on Shimadzu chromatograph with an electron capture detector. Separation of substances was done on a 60 m glass capillary column with DB-5MS UI. The results of analyses (n=560) were usually focused on the total concentrations: dioxin-like PCBs, non-dioxin-like PCBs, common PCBs. Sampling was done at the old (NN 1-9) and new (NN 10-20) landfills. Conclusions: 1. Dumps of municipal waste of Dilijan, both the old and new sites, are active sources of PCBs accumulation and spread; 2. In soil samples from both landfills excess concentrations of PCBs were found in 100% of cases compared to normative level, and the summary concentrations of the latter multiply exceeded the normative; 3. The problems of PCBs-related environmental pollution are of great concern because the emergence and spread of PCBs in nature is not always subject to control and regulation, as the sources of PCBs formation are diverse.

TU142

Understanding children's exposure to perfluoroalkyl acids - a modelling approach

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The exposure to per- and polyfluoroalkyl substances (PFASs) is of high concern due to their observed toxicity in animal studies and associated health impacts found in human epidemiological studies. Adults are mainly exposed to PFASs via ingestion of food, drinking water, inhalation of air and ingestion of dust, whereas for children the exposure to PFASs is largely unknown. This modelling study aimed to elucidate children's exposure to perfluoroalkyl acids (PFAAs) after cessation of breastfeeding. The studied PFAAs were perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS). A dataset from a Finnish child cohort study was utilized, which comprised measurements of PFAA blood concentrations. PFAA concentrations in dust and air from the children's bedrooms and literature data on dietary PFAA concentrations were used to calculate the estimated daily intakes (EDIs) of the studied PFAAs. The EDIs served as input in a one-compartment pharmacokinetic (PK) model that reconstructed serum PFAA concentrations from 1 to 10.5 years of age. The contribution of biotransformation of precursors in the body (indirect exposure) was assessed for both PFOA and PFOS, while for PFHxS this was not possible due to a lack of data on its precursors.\n\nThe model showed that the direct exposure via dietary intake was the major exposure route for the total PFAAs exposure of the Finnish child cohort, while inhalation of air and ingestion of dust were of minor importance, as was the indirect exposure to PFOS and PFOA. The one-compartment PK model reconstructed median PFOA and PFOS serum concentrations well compared to corresponding measured median serum concentrations. For PFHxS, the modelled serum concentrations were consistently underestimated compared to measured serum concentrations. The derived EDIs for PFOA and PFOS for 10.5-year-olds were in agreement with respective EDIs for adults from other studies, while the derived EDI for PFHxS was substantially lower compared to EDIs of PFHxS from the literature. The derived EDIs are compared with tolerable daily intakes from European authorities. The model results show that children's exposure to PFOA and PFOS after breastfeeding is similar to that for adults, which implies a higher health risk for children due to their lower body weight. More efforts should be made to investigate children's exposure to PFHxS including its precursors and to generate improved PK parameters for this compound.

TU143

Use of Regulatory Models in a Refined, Spatially Explicit Pesticide Ecological Aquatic Exposure Assessment

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Recent risk assessments concerning the effects of currently registered insecticides on aquatic species have adopted regulatory aquatic exposure models and scenarios to represent a wide range of species and habitat characteristics. The application and parameterization of these models to date has been largely at a screening level in terms of the conservativeness of assumptions and the spatial specificity of the exposure model inputs and predictions. The best available spatial datasets provide the information necessary to improve the parameterization of regulatory aquatic exposure models such as the PRZM landscape and VVWM receiving water models, and their resulting exposure estimates. To evaluate the practical use of these models and datasets in a national scale refined ecological risk assessment, 100 different species across a range of taxonomic families, occupying static and flowing habitat, were assessed. The static water modeling approach focused on independently evaluating yearly realizations of crop data to represent multiple cropping configurations surrounding small static water bodies. The proximity of crops to water refined the drift exposure and the distribution of soil types and weather refined the runoff potential. The flowing water modeling approach took advantage of a high-resolution catchment network to determine contributing watershed areas for each distinct catchment within a species range. The catchment network and associated flow length and flow velocity attributes determined the lag-times of pesticide loads contributing from upstream catchments and determined the duration of chemical degradation. The flowing water modeling also considered multiple independent years of crop data to generate an ensemble of cropped area realizations in each catchment and upstream watershed. In both the static and flowing habitat modeling, historical pesticide use data was used to estimate the fraction of catchments receiving insecticide applications for each crop group, which was varied for each of the five crop years modeled. This exposure modeling approach resulted in species-specific EEC distributions that could be further filtered according to habitat characteristics (flow rate, water body size), grouped according to contributing watershed characteristics, and mapped explicitly to locate regions within a species range that are likely to experience exposure levels of potential concern.

TU144

ChemTHEATRE promotes utilization of the published or public data of environmental contaminants for chemical exposure and risk assessment

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There is a general trend toward the growing importance of open data worldwide. It appears to be essential that development of scientific data repositories be accelerated. In the field of environmental chemistry and ecotoxicology, a huge number of monitoring data on chemicals in various environmental and biological specimens have been reported in scientific journals. However, comprehensive, public repositories to store such valuable data set of the chemicals do not exist; researchers are forced to spend lots of time and cost in collecting and utilizing the published data, when modelling environmental behavior and fate of, and performing the risk assessment for, the chemicals of interest. Therefore, it is desirable that various stakeholders in the field should work together to improve and promote secondary use of the data. To this end, we have created a platform to register and visualize the monitoring data of environmental contaminants, named 'ChemTHEATRE' (Chemicals in the THEATRE: Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement). To date, data described in more than 66 projects have been registered on the platform. Users can find e-archived chemical concentration data in the environmental and biological specimens each with associated metadata such as sampling date and location, species, and biometrics, in addition to the detailed description of experimental methods. Bridging ChemTHEATRE to AIST-MeRAM (Multi-purpose Ecological Risk Assessment and Management Tool) storing chemical property and/or hazard/toxicity information provides us high accurate and transparent assessment of ecological risk of chemicals. Much effort is currently being devoted to visualizing e-archived data sets, and enhancing available data-model interfaces to simulate global dynamics of chemical pollution, with Finely-Advanced Transboundary Environmental model (FATE), and to promote a series of integrated exposure and effects analyses. It is thus expected that ChemTHEATRE will be not only a dedicated follow-up and forecasting tool of international regulations on pollution control in the light of traceability and responsible-care engagement of chemicals, but also a 'communication theatre' where a variety of stakeholders can improve their risk literacies and develop new projects through open data access.

TU145

SETAC Exposure Modeling Interest Group

C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering

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Epigenetic and Evolutionary Effects of Environmental Stressors on Environmental and Human Health (P)

TU146

Transgenerational Inheritance of DNA Hypomethylation in Daphnia magna in Response to Salinity Stress

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The salinization of freshwaters is a serious ecological issue that has been receiving increased attention by the scientific community. Salinity is a serious threat to freshwater ecosystems and a relevant form of environmental perturbation affecting freshwater biodiversity through, for example, the impairment of organisms' development and fecundity. Environmental change can induce epigenetic marks in organisms, which in turn influence their responses. Although the transgenerational inheritance of epigenetic marks has been experimentally shown or theoretically postulated, this phenomenon remains barely explored in aquatic species. An example of this knowledge scarcity are the epigenetic responses in *Daphnia*, which is a key organism in studies addressing the ecology of freshwater lentic ecosystems, as well as in aquatic toxicology, including within regulatory frameworks. In the present study, we exposed one generation of *Daphnia magna* to high levels of salinity and found that the exposure provoked specific methylation patterns that were transferred to three subsequent non-exposed generations. These transgenerational effects reflected in the hypomethylation of six protein-coding genes (PAXIP1-associated glutamate-rich protein; Small nuclear ribonucleoprotein G; Prefoldin subunit 3; 60S ribosomal protein L36; Coenzyme Q-binding protein COQ10 B, mitochondrial; Uncharacterized protein) with important roles in the organisms' response to environmental change: DNA damage repair, cytoskeleton organization and protein synthesis. Our results bring to light that epigenetic marks are affected by environmental stressors and can be transferred to subsequent unexposed generations. Accordingly, this work highlights the relevance of epigenetics in environmental sciences and the potential role of epigenetic transgenerational inheritance for gene × environment interactions in *Daphnia*.

TU147

The impact of exposure to environmental stressors on global DNA 5-methylcytosine in zebrafish (Danio rerio) embryos during early development

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Aquatic systems are affected by a wide range of stressors including chemical pollutants and alterations in abiotic factors. Some stressors have been shown to affect the epigenome, leading to persistent effects that can manifest long after the exposure period. In vertebrates, embryos undergo extensive reprogramming events during early development, allowing the formation of totipotent cells and avoiding inheritance of deleterious alterations from parental exposures. We hypothesise that the epigenome of developing embryos is particularly sensitive to stressors during these reprogramming periods. This study aims to determine if common environmental stressors disrupt the methylome of zebrafish (*Danio rerio*) embryos during the reprogramming period, and whether this life stage is particularly sensitive compared to other periods. Initially, we generated dose-response curves for a number of toxic metals (copper, silver and methylmercury), abiotic factors (hypoxia and temperature), and 5-azacytidine as a positive control, to determine the physiological effect of these stressors on embryos exposed from 0 to 24 hours post fertilisation (hpf). In parallel, we conducted dose-response curves during a later developmental period (4 to 28 hpf) to investigate the relative sensitivity of embryos when exposures encompass the period of reprogramming compared to those initiated after reprogramming is complete. In initial experiments, we used a LUMA (LUminometric Methylation Assay) of unexposed embryos to confirm the timing of reprogramming in zebrafish under our experimental conditions. We then used the dose-response curves to determine the effective concentrations that caused 50% effect at 24 hpf (EC50 values) for use in further methylation experiments. For metals, using mortality as an endpoint, these values were 75.77μg/l, 25.51μg/l and 63.71μg/l for copper, silver and methylmercury respectively, under our experimental conditions. For abiotic factors, parameters related to developmental delay were used to define effective concentrations, and these were determined as 20% air saturation for hypoxic conditions and 25°C and 31°C for temperature. We are now testing the effects of each stressor on the global 5-methylcyotsine level in blastocyst embryos using LUMA. The data gathered will identify stressors with the potential to affect methylation profiles during the period of reprogramming in zebrafish embryos, which could cause long-lasting health effects via epigenetic mechanisms.

TU148

The fungicide, tebuconazole, causes persistent changes to the DNA Methylome of Zebrafish (Danio rerio)

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Exposure to environmental stressors during early stages of development can impair physiology of sexually mature adults. One mechanism of these intra-generational effects is mitotically stable alterations to the DNA methylome that result in permanent reprogramming of gene expression. Tebuconazole (TEB) is an agricultural fungicide used globally to control pathogenic fungi found in fruits, vegetables, and grains. Exposures of aquatic organisms to TEB occurs when rain closely follows application of the pesticide, causing it to mobilize and result in elevated concentrations in nearby waterbodies. In the current study, zebrafish (*Danio rerio*) embryos were collected prior to 1-hour post-fertilization and exposed to either 0 μg/L (control), 10 μg/L, or 1000 μg/L of TEB. Exposures ran until 24-hours post-hatch (hph) in order to target DNA methylome reprogramming and the entire period of embryogenesis. Embryos were then transferred into clean water containing no TEB to be reared until sexual maturity. This exposure resulted in significant alterations to the DNA methylome and the enzymatic machinery that maintains the methylome. At 24-hph there was a significant increase in mRNA abundance of the enzyme ten-eleven translocase (*tet*) *3* in embryos exposed to 10 μg/L of TEB, and a significant increase in mRNA abundance of DNA methyltransferase (*dnmt*) *3b1*, *tet 1*, *tet 2*, and *tet 3* in embryos exposed to 1000 μg/L. In sexually mature adults, mRNA abundance of *tet 1* was significantly decreased in gonad tissue of males exposed as embryos to 1000 ug/L of TEB. In brain of sexually mature adults exposed as embryos to 10 μg/L of TEB, there was a significant decrease in mRNA abundance of *dnmt 2* and *dnmt 3b4* inmales and a significant increase of *dnmt 3a2* and *dnmt 3b1* in females. In brain of sexually mature adults exposed as embryos to 1000 μg/L of TEB, there was a significant decrease in mRNA abundance of *dnmt 2, dnmt 3b4, tet 1*, and *tet 2* in males, and asignificant decrease of *dnmt 3a1, dnmt 3b2, dnmt 3b4, tet 1*, and *tet 2* in females. Using reduced representation bisulfite sequencing, 16 differentially methylated regions (12 hypomethylated and 4 hypermethylated) were identified in gonads from females exposed as embryos to 10 μg/L of TEB. Analysis is currently being performed to identity loci-specific changes in DNA methylation in gonads from females exposed to 1000 μg/L. Implications of changes in DNA methylation for physiological performance of zebrafish will be discussed.

TU149

The critical window of exposure of CMIT/MIT, a biocide, in zebrafish Danio rerio: Morphological, Behavioral and Epigenetic approach

H. Lee, University of Seoul / School of Environmental Engineering; N. Chatterjee, University of Seoul / Environmental Engineering; J. Choi, University of Seoul / School of Environmental Engineering

Hynho Lee, Nivedita Chatterjee, Jinhee Choi School of Environmental Engineering, University of Seoul, 163 Seoulsiripdae-ro, Dongdaemun-gu, Seoul 02504, Republic of Korea In present study, we aimed to find out the critical developmental stage for exposure(s) of a widely used biocide, CMIT/MIT, (5-Chloro-2-methyl-3(2H)-isothiazoloneand 2-methy-3(2H)-isothiazolone) in the model system, zebrafish (Danio rerio) embryo. We exposed to various developmental stages (0-24h, 24-48h, 48-72h, 72-96h) and endpoint analysis were carried out either at 96 hpf or 10 dpf. At first, we found out the EC values (EC20, EC50 and EC90) from a range of exposure concentrations (based on coagulation endpoint) and EC20 and EC50 doses were chosen for further analysis. We mainly used two endpoints – morphological (96 hpf) and behavioral alterations (at 10 dpf) and to explain the underlying altered phenomena, we used proteomics and global DNA methylation (with its regulating gene machineries). The early developmental period (0-24h) was found as most sensitive window in respect of morphological (tail bending) as well as behavioral (altered locomotion) endpoints. Moreover, significant number of differentially regulated proteins were evident, specifically, in 0-24h of exposure period. Altered global DNA methylation status (hypermethylation at early window and hypomethylation at later windows of exposures) were also evident which were further supported by DNA methyl-transferase gene expressions. Taken together, the current study shows that exposure window is critical for CMIT/MIT exposure and at early life stage is most vulnerable period in zebrafish model. Though the results are preliminary to translate to in human health scenario, nonetheless, these data could serve as risk assessment of CMIT/MIT for human exposure. This work was supported by the Mid-career Researcher Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (NRF-2017R1A2B3002242).

TU150

Epigenetic, transcriptional and phenotypic responses in Daphna magna after short-term exposure to gamma radiation

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Daphniids are a widely-used model organism for detection of environmental stressors. However, radioactive studies have mainly been focused on vertebrates. This study examined a 7-day cobalt-60 gamma radiation exposure on the crustacean *Daphnia magna* with multiple dose rates and sampling points. Investigated endpoints included histopathology, reproduction, lipid and cellular reactive oxygen species (ROS), global methylation, histone modifications (H3K9) and gene expression of key genes within the One Carbon Cycle linked to methylation and functional genes linked to ROS. Global methylation showed an increase with increasing dose rate (control, 0.4, 1 and 10 mGy/h), with changing ROS from increasing at 2-d, stable at 4-d and increasing at 7-d. Functional genes linked to ROS were all down regulated. In response to the increased global methylation, DNMTs were down regulated and the counteracting genes GNMT and TET2 were upregulated. Reproduction showed no direct effect yet Vitellogenin and Methoprene-tolerant genes showed upregulated activity with increasing dose rate indicating increased reproductive response affective after 7 days. However, histopathology showed cell damage and deformations indicating future reduced reproduction. Cumulatively, the results indicate a gamma radiation induced pathway response in *D. magna* transcending from epigenetic to gene to cellular with indication of future reproductive restriction. Longer exposure time may be needed to induce histone modifications and direct reproductive responses, yet, this study provides valuable insight into multilevel gamma ration induced response in an invertebrate.

TU151

Effect of a biocide, CMIT/MIT on epigenetic modification and phenotypic changes in Daphnia magna: A multigenerational study

J. Gim, University of Seoul; N. Chatterjee, University of Seoul / Environmental Engineering; J. Choi, University of Seoul / School of Environmental Engineering

Epigenetic changes in diverse species, including cladocerans, have been reported to be induced by environmental stress factors and could be transgenerationally inherited without direct exposure at the descendant generation (s). Besides, 'Transgenerational epigenetics' is important in terms of inheritance of a modified phenotype and evolution, but it is still in immature stage of exploring in proportion to 'Intragenerational epigenetics'. CMIT/MIT (5-Chloro-2-methyl-3(2H)-isothiazoloneand 2-methy-3(2H)-isothiazolone) is a known biocide widely used in water treatment processes and is highly toxic to freshwater organisms. In this study, we tested the hypothesis that epigenetic mechanisms, if they are operating, are associated with a heritable modified phenotype. To test the hypothesis, we investigated reproduction, behavior, morphological change, growth and DNA damage to evaluate transgenerational toxicity and epigenetic alteration, such as DNA methylation and histone methylation under various exposure scenarios. We identified that there is transgenerational effects in reproduction, behavior, growth and DNA damage and DNA methylation is highly correlated with growth, showing more dynamic and immediate responses to CMIT/MIT than DNA damage. Our results suggest CMIT/MIT exposure has potential for alteration of epigenetic mechanism, however, more detailed study, such as, gene specific DNA methylation and ChiP-seq studies would needed to understand the roles of epigenetic alteration in inheritance of modified phenotype.

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Keywords : Epigenetic modification, Transgenerational toxicity, CMIT/MIT

TU152

Histone methylation associated transgenerational toxicity in the nematode Caenorhabditis elegans

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Epigenetics, phenotypic characters without modification of DNA sequence, possess reversibility as well as the heritable transgenerational transfer of epigenetic marks. The aim of the present study was to evaluate the role of histone methylation in transgenerational response to environmental chemical exposure in the nematode, *Caenorhabditis elegans*. C.elegans were exposed to environmental chemicals, such as, biocide and crude oil, and transgenerational toxicity was investigated in *C. elegans*on the exposed (P0) and three consecutive unexposed generations (F1-3). We found true trans-generational inheritance of toxicity caused by the crude oil in the unexposed generations. Next, we examined response to chemicals exposure using histone methylation related mutants, such as, *mes-2* (H3K27 di-/trimethyl transferase), *met-1* (H3K36 and H3K9 trimethylase), *met-2* (H3K9 and H3K36 trimethylase), *jmjd2* (H3K9/H3K36 demethylase), *set-2* (H3K4 methyltransferase), *spr-5* (H3K4me2 demethylase) and *utx-1* (H3K27 demethylase). Decreased methylation of histone H3 marks (i.e., H3K9) was subsequently found in the oil-exposed generation, however a heritable diminution in reproduction did not occur in the H3K9 histone methyltransferases (HMT) mutant, *met-2(n4256)*, suggesting a potential role for the HMT of H3K9 in transgenerational toxicity. Our overall results suggest that chemical toxicity could be heritable and histone methylation may be associated with the transmission of the inherited phenotypes. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R& D Program' (2017001370001). ***Keywords****: Caenorhabditis elegans*, transgenerational toxicity, epigenetics, histone methylation

TU153

Epigenetic-associated genes in Caenorhabditis elegans are potential toxicity biomarkers for metals and per- and poly-fluorinated alkyl substances

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Cancer, autism, obesity and asthma are epigenetic-associated disorders and syndromes. While numerous studies have correlated exposure to pollutants such as metals and persistent organic pollutants, with epigenetic changes, it remains a poorly understood area. This study aimed to explore the possible transcriptional deregulation of epigenetic-associated genes in *Caenorhabditis elegans* exposed to per- and poly-fluorinated alkyl substances (PFAS) and metals during larvae development, from (L1) to pre-adult (L4) stage. *C. elegans* was the first animal whose genome was fully characterized and is a valuable *in vivo* model for PFAS and metal toxicity studies. PFAS and metal exposure of *C. elegans* resulted in changes in expression patterns of epigenetic-associated genes. *C. elegans* exposure to PFAS, resulted in altered expression of genes encoding for histone methyl- and acetyl- transferases, as well as histone deacetylates and demethylases. However, *C. elegans* exposure to a metal mixture and soil leachate comparatively altered the transcription of fewer epigenetic-associated genes, indicating differential effects on epigenetic markers. The present results suggest that the transcriptional responses of epigenetic-associated genes, makes them valuable as potential biomarkers. The present study motivates further investigations into the mechanisms associated with epigenetic effects of pollutants in the environment.

TU154

Genetic, epigenetic, and developmental toxicity of Chironomus riparius raised in metal-contaminated field sediments: A multi-generational study with arsenic as a second challenge

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Jeongeun Im, Nivedita Chatterjee, Jinhee Choi School of Environmental Engineering, University of Seoul, 163 Seoulsiripdae-ro, Dongdaemun-gu, Seoul 02504, Republic of Korea Ecotoxicity tests conducted under well-controlled lab conditions often do not reflect the real environmental conditions. To this end, we designed an ecotoxicity test using an aquatic midge, *Chironomus riparius* (*C. riparius*), raised in metal-contaminated field sediments (MCFS), which reflect the real environmental conditions, for five consecutive generations (F0–F4) followed by a toxic response to arsenic exposure (as a second challenge). The toxic responses (i.e. DNA damage, DNA methylation, stress response gene expression, and mortality) were compared to those organisms reared in lab sediments (LS). Under the MCFS condition, increased adult emergence of the larvae was observed for the first two generations (F1 and F2), while a decreased tendency was evident thereafter (F3 and F4) compared to that of F0. When comparing *C. riparius* raised in MCFS or LS exposed to arsenic, increased sensitivity (declined survival) was observed in the larvae from F2, whereas, such a tendency was not present in F4 of the MCFS midges, indicating a possible physiological adaptation. Increased DNA damage was observed in the MCFS-exposed organisms compared to the those exposed to LS, particularly at F0. Arsenic exposure induced hypermethylation at F0 and, in contrast, hypomethylation at the later generations (F2, F4) in the MCFS-exposed organisms. Global DNA methylation results were supported by related methylation enzymatic gene expressions. Moreover, alterations in oxidative stress related to gene expressions showed that significant oxidative stress and perturbation of glutathione reserves occurred under the MCFS and the subsequent arsenic exposure conditions. Overall, our results suggest that multigenerational rearing under MCFS conditions resulted in physiological adaptation of *C. riparius* to metal exposure, specifically at later generations, which in turn modulated its response to arsenic stress through possible genetic and epigenetic mechanisms. This work was supported by the Mid-career Researcher Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (NRF-2017R1A2B3002242).

TU155

Toxicity of neonicotinoids and other pesticides to Chironomus riparius: pulse, chronic and multigenerational tests

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Neonicotinoid insecticides (NNIs) were included in the EU watch list substances for emerging water pollutants (Decision 2015/495) and recently, three of them have been banned from outdoor use. They are very persistent contaminants in soils, with DT_{50} reaching thousands of days. From soils, NNIs leach to water bodies due to their solubility and are found extensively around the globe. Additionally, they are toxic to beneficial organisms, especially insects. In our study, we investigated the toxicity two NNIs and other pesticides to the aquatic insect *Chironomus riparius* (Diptera: Chironomidae). The types of tests used include growth, emergence and second-generation growth tests with both chronic and pulse exposures. Insects were exposed in water-sediment systems to the neonicotinoid thiacloprid as the main compound. Thiamethoxam, propiconazole, triasulfuron, azoxystrobin and MCPA were added subsequently to test the toxicity of mixtures. Concentrations of the single compounds never exceeded $2\mu g\ L^{-1}$ and $10\mu g\ L^{-1}$ in the chronic and pulse exposures, respectively. Although survival was not affected, every single combination of the pesticides tested caused an inhibition in the growth of *C. riparius* over 10d tests when compared to unexposed larvae. A mere $0.5\mu g\ L^{-1}$ of thiacloprid caused a significant inhibition of growth. During the emergence experiment, larvae were exposed to chronic or pulse concentrations of the same combinations of pesticides as in the growth tests. Adult emergence, sex ratio and number of eggs per female were recorded. In general, pulse exposures affected the emergence and sex ratio of adult chironomids more than the chronic exposures. Emerged adults of each pesticide treatment were transferred to specific breeding cages for oviposition. After hatching, larvae resulting from parents with different exposure history were grown in clean and thiacloprid-contaminated scenarios. However, the results showed no major differences in the growth among any of the second-generation larvae despite the conditions in which the first generation were reared. Overall, the present study adds important information about the toxicity of NNIs and pesticide mixtures to aquatic insects, raising questions about the importance of pulse exposures in the risk assessment of pesticides. Moreover, it highlights the need of more studies on the toxicity of pesticides to several generations of insects to establish a link with the real status of insect populations.

TU156

Response to AHR ligands in liver slices cultured from naïve and pre-treated chicken embryos; does epigenetics play a role?

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Lipophilic environmental contaminants such as dioxin-like compounds (DLCs) and polycyclic aromatic hydrocarbons (PAHs) can be found in high concentrations in the eggs of wild birds. We are interested in how this early-life exposure to contaminants affects sensitivity to re-exposure later in life, and whether sensitivity to re-exposure is regulated by epigenetic mechanisms. In the current study, the DLC, tetrachlorodibenzo-*p*-dioxin (TCDD), or the PAH, benzo[*k*]fluoranthene (BkF) was injected into fertilized chicken eggs prior to incubation. At embryonic day 19, livers were harvested and slices of the tissue were grown in culture. These liver slices were then re-exposed to graded concentrations of each of the test chemicals. Both BkF and TCDD were associated with dose-dependent induction of several genes associated with the aryl hydrocarbon receptor (AHR) response pathway including *cyp1a4, cyp1a5,* and *ahrr* (but not *ahr* or *arnt*). A large degree of variability was observed in the responsiveness of liver slices cultured from different individuals. Interestingly, liver slices from individuals exposed to BkF *in vivo* were more sensitive to induction of *cyp1a4*, *in vitro*. This did not appear to be a carry-over effect from the initial egg injection; PAHs such as BkF are known to be rapidly metabolised by avian embryos, and the levels of *cyp1a4* mRNA expression in the untreated liver slices suggested that any induction due to the *in vivo* treatment was transient. Exposure to BkF was also associated with small but significant increases in methylation of the CYP1A4/5 shared promoter. Ongoing work is examining the role of histone acetylation in the response to re-exposure to AHR ligands at this locus. Epigenetic marks associated with genes involved in xenobiotic metabolism may be useful as biomarkers describing an association between early life exposures to environmental contaminants and sensitivities to subsequent exposures later in life.

TU157

Genotoxicity of active compounds mesotrione and s-metolachlor and their commercial formulations in Vicia faba L.

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Pesticides is a task of major concern and Brazil is the country that consumes more pesticides in the world since 2011. Corn is the second culture more cultivated in Brazil and for weeds control some herbicides are used. Amongst them are the herbicides based on the active principles mesotrione and s-metolachlor. These substances can persist on the environmental and contaminate non-target organisms. Therefore, it is of interest to study the toxicological risk of such compounds. Hence, the aim of this study was to investigate the effects of active pure compounds mesotrione and s-metolachlor and their respective commercial formulations through *Vicia faba* L. (broad bean) micronucleus bioassay. The experiment was plotted following the 48 h-protocol of ISO 29200. A range of dilutions of the active principles and the commercial formulations was tested from 1.56 to 50 mg L^{-1}. The Hoagland's solution was used as negative control and Maleic hydrazide as positive control. The micronuclei frequency and mitotic index were recorded in the F1 cells of the secondary roots of *V. faba* and expressed in each 1,000 cells evaluated. A total of 6,000 cells was recorded (1,000 cells per root tip and 6 roots per treatment). For the pure compounds, a decrease on mitotic index was observed. Mesotrione significant reduced ($p< 0.05$) the frequency of cell in division in concentrations > 12.5 mg L^{-1}. For S-metolachlor a significant reduction ($p< 0.05$) was noticed only in the highest concentrations (50 mg L). In addition, the frequency of micronucleus increased significantly ($p< 0.05$) in the treatment with 3.125 mg L^{-1} of Mesotrione and 6.25 to 25 mg L^{-1} of S-metolachlor. On the other hand, the treatments with the commercial formulation containing the same concentrations of active compounds do not damage plant DNA. All together, the results demonstrate that in commercial formulations, combined effects of the pure compounds and the solvents or other substances could occur and interfere on the genotoxic potential of the active compounds. Moreover, the cytotoxicity of S-metolachlor and Mesotrione was in the same order of magnitude, while Mesotrione alone is more genotoxic than S-metolachlor.

TU158

Phenotypic and genotypic adaptations in a riverine green alga (Chlorophyceae, Selenastraceae) as a response to long-term exposure to chemical stress

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Abstract Herbicide pollution is a common problem in agricultural streams due to their extensive use and their unwanted effects on non-target species inhabiting these systems. *Selanastraceae* is a family of green algae that are particularly dominant in freshwater, making them a key component in the base of the food chain and an important part of the oxygen production. Therefore, it is crucial from a conservation point of view to accurately monitor the ecotoxicological impact of herbicides on this group of algae, not only on a short-term scale (e.g. days or weeks), but also in a long-term perspective (e.g. months and years). In this work, phenotypic and genotypic differences are studied between different strains of a population of *Kirchneriella* (Chlorophyceae, Selenastraceae) isolated from a stream polluted by herbicides over a 20-year period (Skivarpsån, SE Sweden). According to mixture toxicity modelling performed on data obtained from the Swedish pesticide monitoring program, the main drivers of algal toxicity in this river are the herbicides diflufenican (carotenoid synthesis inhibitor) and isoproturon (PSII inhibitor). Three strains of the isolated algae are examined in this study: two strains from the field that have been exposed to different levels of herbicides and one laboratory strain that has never been exposed to herbicide pollution. The taxonomy of this algae is investigated by light microscopy and DNA barcoding analyses (chloroplast 23S rRNA gene). Phenotypic differences between the strains are examined as impacts on growth rate, cell size, photosynthetic activity (chl fluorescence measurements), pigment content and sensitivity to the specific herbicides mentioned earlier (EC50s). Genotypic differences are studied as single nucleotide polymorphisms thought to be induced through exposure to diflufenican and isoproturon on pds and psbA genes via PCR amplification, cloning and sequencing. The results obtained from this project will contribute to a better understanding of genetic and phenotypic adaptations in green algae as a response to herbicide pollution (evolutionary toxicology) and their implications for ecological functions.

TU159

Can pesticide stress affect genomic patterns of freshwater invertebrate organisms? A pilot study in agriculture areas in Southern Sweden

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The expansion and intensification of agricultural areas and the associated chemical pollution remains one of the most important stressors to aquatic ecosystems worldwide. Once chemicals from agricultural activities enter aquatic ecosystems, they may cause unwanted ecological consequences from the molecular to the ecosystem level, impacting the ecological health of aquatic ecosystems. Invertebrate communities represent one of the Biological Quality Elements according to the EU Water Framework Directive. They are therefore extensively used as biological indicators to assess water quality. However, a thorough understanding whether pollution from agriculture drives genome-level modifications of invertebrate freshwater organisms is still absent. We therefore used approaches from evolutionary ecotoxicology to address impacts of complex pesticide pollution on the genome of natural populations of the shredder invertebrate *Gammarus pulex*. The study was conducted in the southern part of Sweden, where the main agricultural activities take place. The area is therefore recognised as a hotspot for pesticide application and is part of Sweden's national pesticides monitoring. Three stream ecosystems were sampled and *G. pulex* was sequenced using 2bRAD technique. Our study informs on alterations of genomic regions and responses of aquatic invertebrates to complex pesticide pollution in distinct agricultural landscapes as well as gathering critical environmental information about mechanisms driving genomics changes in freshwater populations.

Complex Mixtures in the Environment: Monitoring, Fingerprinting and Assessment (P)

TU160

A Prospective Approach for Assessing Chemical Mixtures in River Catchments with Diverse Land Uses

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Field-based ecological risk assessments incorporate risks from chemical mixtures and a myriad of stressors because ecosystems are continuously exposed to a wide-array of contaminants and nonchemical stressors. Considering the large numbers potential combinations of mixtures and stressors, this problem could seem insurmountable. We demonstrate that such combinations can be simplified by 3 land-use related chemical emission scenarios: agriculture, domestic, and urban. We applied a tiered methodology to assess the implications of each of the scenarios via a quantitative model. The results showed land use–dependent mixture exposures, clearly discriminating downstream effects of land uses, with unique chemical "signatures" regarding composition, concentration, and temporal patterns. Associated risks were characterized in relation to the land-use scenarios. Comparisons to measured environmental concentrations and predicted impacts showed relatively good similarity. The results suggest that the land uses imply exceedances of regulatory protective environmental quality standards, varying over time in relation to rain events and associated flow and dilution variation. Higher-tier analyses using ecotoxicological effect criteria confirmed that species assemblages may be affected by exposures exceeding no-effect levels and that mixture exposure could be associated with predicted species loss under certain situations. The model outcomes inform various types of prioritization to support risk management, including a ranking across land uses as a whole, a ranking on characteristics of exposure times and frequencies, and various rankings of the relative role of individual chemicals. Though all results are based on in silico assessments, our land use–based approach yields useful insights for simplifying and assessing potential ecological risks of chemical mixtures and can therefore be useful for catchment-management decisions. grossly confirmed by results of similar Europe-wide assessments made by the SOLUTIONS project and can, therefore, be useful for evaluating chemical safety assessment as well as catchment-management decisions.

TU161

Non-targeted analysis of xenobiotics in fish muscle

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The complexity of the occurrence and toxicity of emerging contaminants in aquatic environments is remarkable. On the one hand, the identification and quantification of unknown pollutants in the media is a challenging work, due to the high amount of anthropogenic compounds released to the environment. Moreover, the different physicochemical properties of the compounds and thus, their degradation and bioaccumulation processes affecting aquatic organisms are often missing due to a lack of analytical procedures to measure emerging compounds in biota. The objective of this study was to optimize the extraction and purification steps to analyse xenobiotics in a biological tissue from a non-target or suspect analysis point of view. In this sense, the optimization was performed in fish muscle through liquid chromatography coupled with a high resolution mass spectrometer on a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap equipped with a heated ESI source (Thermo, CA, USA). A selection of more than 200 compounds, including personal care products and pharmaceuticals, consumption products (additives, detergents), industrial compounds (perfluoroalkyl substances, plasticizers), and pesticides was used for method optimisation. For extraction, focused ultrasound solid-liquid extraction (FUSLE) and Precellys homogenization were tested using acidified acetonitrile and salting out effect. The clean-up of fish fillet extracts was done using different solid phase separation techniques as: (i) combination of normal phases as alumina, silica and Florisil; (ii) C18 and polymeric reverse phases; (iii) anionic and cationic exchange mixed-mode cartridges; and (iv) specific cartridges for proteins and lipids elimination. The best approach was chosen as the best consensus between the lowest matrix effect and the most efficient recovery of the larger mount of target compounds. Analysis of the extracts was performed in positive and negative mode in the Full scan data-dependent MS2 discovery acquisition mode. Apart from accuracy and precision of the method, limit of identification was calculated for the target compounds. Finally, the optimized method was applied to real samples from field samplings in the Biscay coast and commercial species acquired in local supermarkets, performing a full suspect analysis with a database of more than 40,000 compounds from the NORMAN list.

TU162

Assessment of Southwestern Atlantic pollution: POPs and Chlorpyrifos in seabirds from the south east of Argentine

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Persistent Organic Pollutants (POPs), such as Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are anthropogenic chemicals used for industrial and agricultural purpose; they were banned years ago and are regulated under the Stockholm Convention. POPs occurrence in the environment is of great concern due to their effects on organisms. Birds feathers constitute a useful non-destructive tool for POPs detection. There is scarce information about levels of these compounds in birds from the southern hemisphere. The aim of this work was to determine levels of POPs and chlorpyrifos in feathers of three seabirds wintering in Southwestern Atlantic, Olrog's gull (*Larus atlanticus*), Black-browed albatross (*Thalassarche melanophris*) and Cape petrel (*Daption capense*) and the relationship with sex and spatial distribution. POPs and chlorpyrifos were quantified by gas chromatography with electron capture detector (GC-ECD). All species showed a predominance of chlorpyrifos followed by OCPs, PCBs and PBDEs. This denotes the importance of agricultural activity in the region. Particularly, the Olrog gull presented the greatest contaminant levels (X=180,97ng/g), according to its distribution, being related to coastal areas, mainly associated to marshes. Regarding to sex differences on pollutants load, only females of Olrog gull showed lower levels than males (X=100,27; 288,56ng/g respectively), probably because of maternal transfer. Differences were also detected among pelagic species. Pollutants levels were higher in Cape petrel (X=151,63ng/g) than in Black-browed albatross (X=80,47ng/g) possibly as a consequence of higher lipid mobilization during migration. In all species, HCHs dominated OCPs composition, followed by endosulfans (α-/β->1). Among PCBs, tri-, tetra- and penta- CB were the most abundant congeners in feathers of Olrog gull and Black-browed albatross, while CB 156, CB 180 and CB 183 were the predominant PCBs in Cape petrel. The same PBDEs pattern was observed in both pelagic species, being BDE-47 and BDE-99 the predominant congeners followed by BDE-100; however, BDE-100 was the only PBDE detected in feathers of the Olrog gull. These results are similar to worldwide trends found in birds feathers. Therefore, the organic pollutants occurrence in birds from the southern hemisphere deserves more attention and long-term monitoring programs are suggested to reduce their incorporation to aquatic ecosystem.

TU163

Using targeted and non-targeted screening analyses to assess bioaccumulation and toxicity of produced water components on copepod populations in the North Sea

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Produced water from the oil and gas industry represents the largest direct discharge of effluent into the marine environment worldwide, with total releases from activities on the Norwegian continental shelf alone contributing with > 30,000 tons of organic compounds of potential environmental concern. Due to rapid dilution and (bio)degradation, impacts on local populations of marine species is expected to be minimal. However, this hypothesis needs verification. In the current study, we developed and demonstrated a novel sample preparation technique that allows analysis of target and non-target body residue (GC-MS/MS, GCxGC-HRMS), metabolomics (NMR) and lipidomics (LC-qTOF-MS) profiling of the same sample material. This enables direct comparison and correlations between bioaccumulated produced water components and effects on biological systems. The method was applied to copepods sampled from four different locations in the vicinity of North Sea oil producing platforms which are continuously emitting produced water to the marine environment. From the methods used here, multivariate analysis of the PAH profile and of the lipidome data allowed for differentiation of the four copepod populations, whereas the metabolome data showed no differentiation.

TU164

Characterization of Bioavailable Environmental Pollutant Patterns in Marine, Estuarine and Freshwater Sediments

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Sediments are well known sinks for a wide range of hydrophobic organic contaminants and might act as a long-term source of pollution for aquatic ecosystems. Although the environmental relevance of sediments has been acknowledged, the assessment of their risk is often biased when applying exhaustive extraction methods and normalization to their fraction of organic carbon. This problem can be tackled by equilibrium passive sampling, which represents the bioavailable fraction of sediment contamination. Sediment samples collected in Sweden (n=4), the Saar river catchment (Germany) (n=10), the European Arctic (n=9) and Australia (n=4) were equilibrated with silicone-coated jars by horizontal rolling for three weeks. The toxicological characterization of the samples was established by reporter gene bioassays and revealed distinct patterns for a range of endpoints (Jahnke et al., Environ. Sci.: Processes Impacts 2018, DOI: 10.1039/c8em00401c). As the first step of the chemical characterization, the extracts were analyzed by a gas chromatography-high resolution mass spectrometry (GC-HRMS) instrument equipped with an electron ionization (EI) source (Q-Exactive GC, Thermo Scientific). The analysis focused on a target list of 160 compounds covering a wide spectrum of chemical classes. The preliminary results revealed specific contamination patterns. As an example, most of the PCBs were ubiquitous at all the sampling points. However, the site of Ålöfjärden (Sweden) was found to be a hot spot of PCB contamination, having concentrations three orders of magnitude higher than all the other sampling sites. Moreover, site-specific contaminants were found in Saar river samples, being the only ones containing the musk compound Phantolide, whereas other musk fragrance materials such as Galaxolide and Tonalide were detected at all sampling spots. As a next step, the extracts will be investigated for a comprehensive suspect list including recently discovered emerging contaminants, which will be followed by non-targeted approaches to identify site-specific compounds in addition to the ones that are ubiquitous, providing a basis of chemical characterization of marine, estuarine and freshwater sediments.

TU165

APEX - Systematic use of contaminant data from apex predators and their prey in chemicals management

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How can environmental monitoring data support the safe use of substances and products in Europe? The aim of the EU funded LIFE APEX programme (2018-2022) is to improve systematic use of chemical monitoring data from apex predators and prey for protecting human health and the environment. More specifically, LIFE APEX responds to needs of regulators for specific regulatory applications in relation to REACH and the Biocidal Products Regulation.

Chemical monitoring data from apex predators (e.g. raptors, otters, seals) are of particular value; their position at the tops of food webs means they act as sentinels to reveal harmful substances, in terrestrial, freshwater and marine environments. When combined with data from selected prey (e.g. fish), apex predator data can deliver useful quantitative information on the persistence and bioaccumulation. LIFE APEX will make use of novel analytical methodologies that allow for screening of several thousands of chemical substances in each sample and prioritization of frequently occurring pollutants and their mixtures. LIFE APEX involves making better and more cost-effective use of chemical monitoring data from the large, valuable but under-used resource of environmental samples in Europe's Environmental Specimen Banks, Natural History Museums and other research collections. The objectives of LIFE APEX are: 1. To demonstrate four novel, regulatory applications of chemical monitoring data, specifically: a) to detect presence of chemical contaminants in the environment; b) to facilitate selection of most relevant substances for further hazard assessment; c) to assess impact and effectiveness of substance risk mitigation measures; d) to define predominant chemical mixtures in the environment. 2. To support and sustain regulatory take-up of these applications, specifically: a) to assess relevant resources and capacities for replication and transfer and engage key partners; b) to enhance quality assurance of sampling, processing, archiving and analysis of food web samples (and resulting data); c) to enhance availability and access to relevant apex predator and prey samples and related chemical monitoring data and the comparability and interoperability of this data. 3. To replicate and transfer LIFE APEX approaches and methods with partners across Europe. 4. To disseminate and communicate the LIFE APEX approaches and methods and in particular optimize uptake by regulators and industry.

TU166

Spatial and temporal trends in e-waste related organic pollutants in a developing economy - A pilot study

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A considerable amount of research has been carried out mapping the occurrence and levels of e-waste related pollutants in the environment in developing regions known as major recipients of e-waste from industrialized countries. However, little is known about levels and trends of such pollutants in emerging economies with increasing domestic consumption of electronic equipment, yet lacking adequate formal disposal and recycling systems. A pilot study was conducted in Tanzania to inform upcoming sampling strategies within a larger research project (AnthroTox). A goal of this project is to assess spatial and temporal trends of e-waste related legacy persistent organic pollutants, emerging organic contaminants, and selected metals in air, soil and dated sediment cores. The pilot study was conducted to assess the presence of e-waste related organic pollutants in soil samples collected at 12 sites suspected to be highly polluted and/or potentially connected to e-waste related activities. The samples were analyzed for 32 PCBs, 25 PBDEs, 13 new brominated flame retardants (nBFRs), 39 short and medium chain chlorinated paraffin (S/MCCP) homologue groups, and four Dechloranes. S/MCCPs were found in highest levels, with mean concentration of 480 ng/g dry weight (dw) for both SCCPs and MCCPs (range 18–1900 ng/g dw, and 4–2100 ng/g dw for SCCPs and MCCPs respectively). The highest level of SCCPs were found at an unofficial landfill, while the highest levels of MCCPs were found at an e-waste handling facility. nBFRs had mean concentration of 53 ng/g dw, with a range of 1–340 ng/g dw. The highest levels of nBFRs were found at an old landfill, followed by an e-waste handling facility (126 ng/g dw). Mean concentration of PBDEs was 50 ng/g dw (range 1.7–200 ng/g dw). The highest levels were found at an old landfill currently used as farmland, followed by an e-waste handling facility (72 ng/g dw). The mean concentration of PCBs was 25 ng/g dw (range 0.2–170 ng/g dw), with the highest levels found at an old landfill. Dechloranes had the lowest levels, with mean of 3.8 ng/g dw (range 0.1–10 ng/g dw). The highest levels were found in farmed soil adjacent to an old landfill. The levels of pollutants and the relative amount of compound groups were highly variable from site to site. At some locations, the levels found were comparable to heavily industrialized areas, and e-waste dumping sites in Asia, making Tanzania interesting for further research.

TU167

The evaluation and selection of techniques for non-directed analysis of xenobiotics in water using LC-HR-Q-Orbitrap/MS

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New technological and operational approaches are needed to screen the most toxic contaminants in the environmental and to get insights about their sources, usages and effects on organisms, including human health. The transition from target analysis to non-target analysis through suspect analysis has become a rising field. Recent developments in data-independent/data-dependent acquisition (DIA/DDA) workflows in LC or GC-HRMS techniques allow the identification of hundreds of chemicals in a single injection maximizing information collected (i.e. molecular formula, isotopic profile, fragments, retention time). These approaches can be applied to screen many chemicals, providing information on the most relevant occurring ones that can be prioritized for further quantitative analysis, reducing the impact of a-priori taken decisions. Therefore, the objective was to implement a method for the suspect/non-target screening (NTS) of organic xenobiotcs in water, including wastewater treatment plant effluent, estuarine and riverine water. First, the extraction step (using a large volume solid-phase extraction (LVSPE50) device with a sorbent combination of 6 g Chromabond HR-X (Macherey-Nagel), 2 g Sepra ZT-WAX (Phenomenex) and 2 g Sepra ZT-WCX (Phenomenex) phases) was validated for 35L of water in terms of extraction efficiency, matrix effect and reproducibility of more than 200 compounds. Those include a wide set of known compounds including pharmaceuticals, personal care products, plasticizers, etc. which were analysed on positive or negative mode in the Full MS ddMS2 discovery acquisition mode in a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific Q Exactive quadrupole-Orbitrap mass spectrometer (Thermo, CA, USA). This acquisition mode allows the fragmentation of peaks with the highest signals, without any previous list, thus, the most abundant. Matrix effect correction was evaluated using standard additions before and after extraction. Other quality control parameters such as the limit of identification were also calculated. The validated method was applied for the suspect screening of 40054 compounds included in the NORMAN suspect list and the NTS of halogenated compounds on effluent of Galindo WWTP at different periods of the year, estuarine water in the Nerbioi-Ibaizabal, and different rivers from the Biscay province (Basque Country, Spain). Both suspect and NTS workflows were run using the Compound Discoverer software (Thermo, Ca, USA).

TU168

Ultra-trace analysis of nitrosamines in drinking water using triple quadrupole GC-MS/MS advanced electron ionisation

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Nitrosamines are emerging drinking water contaminants linked to cancer by either ingestion or inhalation. Due to their high toxicity, nitrosamines are considered as priority pollutants and various countries around the world have already introduced maximum acceptable levels such as those highlighted in EPA 521 method for compounds such as NDMA, NDEA, NDPA, NDBA, NPYR and NPIP. Nitrosamines are considered toxic in drinking water at very low which meaning that method sensitivity is of highest importance. An additional challenge for nitrosamine analysis is selectivity in matrix as these compounds have low molecular weights, fragment easily and often cannot be quantitated from their molecular ion species. Poor selectivity and sensitivity can lead to false positive detection and inaccurate quantification of these compounds however gas chromatography (GC) coupled to triple quadrupole mass spectrometry (MS) provides extra selectivity and sensitivity compared to single quadrupole technologies. Current methods utilize SPE analyte concentration coupled with large volume injection (LVI) to achieve the required levels of sensitivity. However, LVI introduces more contamination into the GC-MS/MS system leading to increased frequency of GC consumable changes, instrument maintenance and source cleaning lowering productivity and increasing the cost per sample. Here the use of a novel AEI high performance electron ionization (EI) source in conjunction with a new triple quadrupole mass spectrometer provides the sensitivity and selectivity to analyze nitrosamines in drinking water at low part per trillion (ppt) levels with low liquid injection volumes. In this work the Thermo Scientific™ TSQ 9000™ GC with Advanced Electron Ionization Source mass spectrometer was evaluated for nitrosamines selectivity, sensitivity, recovery, repeatability and linearity of response.

TU169

Characterisation of pesticide mixtures and their effects on freshwater communities in the Aconcagua River, Chile

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Pesticides can affect non-target freshwater organisms and therefore alter the structure and/or function of aquatic ecosystems. In freshwater ecosystems, pesticides occur as complex chemical mixtures, whose effects have been shown greater than single compounds effects. Although pesticides are a well investigated group of chemicals, many uncertainties regarding mixture effects on aquatic ecosystems remain unknown. Here, we conducted a risk characterisation of pesticide mixtures and assessed their effects on freshwater communities in the Aconcagua River, Chile. Four specific objectives were established: (1) to assess the pesticide status of the Aconcagua River, (2) to characterise the biological status of freshwater communities, (3) to describe major correlations between pesticide and biological status, (4) to conduct predictive analysis using concentration addition and independent action approaches. Chile is one of the top food producers in South America and the Aconcagua River is one of the most important rivers in the country, which hosts mining activities, intensive agriculture and several wastewater treatment plants along its basin. Nine sampling sites were selected (three reference, three tributaries and three river sites) in order to characterise the river basin chemically and biologically. Two different analytical approaches were used to describe the pesticide status along the river: (i) grab surface water samples (2 L) were collected and analysed following the EPA method 508 by GC-MS, and (ii) 50 L were filtered *in situ* using large volume solid-phase extraction (LVSPE) and used for LC-HRMS analysis, also, further bioanalysis will be conducted. Bacteria from sediment, biofilm, macroinvertebrates and environmental DNA were collected in order to conduct diversity analyses and determine the biological status along the river.

TU170

Contribution of vegetation-related chemical fingerprints for the chemical composition of river waters

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River water contains large number of plant metabolites that possibly pose risk on human being, when the water is used for human consumption. Some of these metabolites are species-specific while others are common to all or most plant species. The majority of metabolites released by plant to river water are unknown. Several approaches have been implemented for the identification of chemical composition, mainly anthropogenic origin, of river waters. On the other hand, large number of chemical signals detected in river water was left unidentified. Some or most of these unidentified components might be contributed by natural or agricultural vegetation. Thus, an alternative approach has to be implemented to investigate the chemical contribution from vegetation. To address these, we analysed extracts from widespread natural plants (e.g *Allium ursinum*, *Fallopia japonica*) and agricultural crops (e.g. wheat, rapeseed, corn, potato) and water from adjacent rivers by non-target screening using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Among the detected nontarget peaks in plant extracts, we could discriminate peaks showing up in extracts from one plant (plant specific peaks) but not in the remaining plants and background reference river waters. For each plant, from several thousands of plant metabolites, specific peaks with intensity above 10^5 were distinguished, which were used for annotating peaks from water samples. Accordingly in river samples adjacent to the specific plant stocks, several dozens of such plant-specific peaks could be detected (e.g., 68 for *Allium ursinum* and 227 for rapeseed). The majority of these peaks elute at low retention times indicating high hydrophilicity. MS/MS spectra for most pairs of overlapping peaks confirmed that they were the same compounds. Thus, our study indicates that plants are the sources for the observed overlapping peaks in stream samples. Current activities focus on the identification of these compounds and evaluation of their potential toxicity.

TU171

An automated approach for the determination of gasoline range organics in water using gas chromatography coupled with static headspace sampling

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Gasoline Range Organics refer to hydrocarbons with a carbon range from C6-C10 that have boiling points ranging from 60^0 to 170^0 Celsius. These chemicals are frequently present in the environment especially in soils and ground water and the source of contamination is usually human errors or accidents (such as oil spills) that occur when handling, storing or transporting oil and oil products. If GRO are detected in soils or water, the level of contamination needs to be determined by using quantitative analytical methods. Often the method of choice is head space sampling techniques coupled to gas chromatography and mass spectrometry and/or flame ionisation detectors. In this work, the Triplus Plus 500 valve-and-loop static headspace coupled with a Trace 1310 gas chromatograph and an ISQ 7000 mass spectrometer were employed to develop an automated method for quantitative assessment of GRO in water. Chromeleon chromatography data system was used to acquire, process and report data. The performance of the method was assessed and various analytical parameters investigated. The data presented in this study demonstrates the method performance and reliability in addition to automated data processing and reporting capabilities.

TU172

Profiling Environmental Mixtures in Water using GC/Q-TOF

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The traditional approach to monitoring organic contaminants involves analyzing a defined number of target compounds by mass spectrometry with the instrument operated in a selected data acquisition mode for targeted analytes. However, there is evidence that such an approach may significantly underestimate the exposure and risk of pollutants, compared to a more comprehensive broad-scope targeted and untargeted screen. With the plethora of chemical mixtures present in the environment, the ability to measure as many of them in an intargeted fashion is important. Recent advances in mass spectrometry allows an increased scope of analysis when using high resolution accurate mass instruments operated in full spectrum acquisition mode. Accurate mass information enhances the amount of detail in the information collected and allows for the determination of both targeted and non-targeted components using libraries, databases and fragment predictors. This study will present a workflow combining targeted quantitation, a broad scope suspect screen using curated databases and libraries as well as non-targeted screening using surface water samples collected in Sacramento-San Joaquin Delta in Northern California with a high-resolution accurate-mass GC-QTOF instrument. Untargeted screening was enhanced and facilitated with the use of a novel low energy EI mode that allowed greater molecular ion detection and hence easier identification in these water samples. The study detected several compounds not initially looked at during the targeted analysis that where present at high abundances in the samples.

TU173

Analysis of unknown Organic Compounds in Reservoir-Water by using Comprehensive Two-Dimensional Gas Chromatography/Time-of-Flight Mass Spectrometry

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A high throughout screening method, which used by comprehensive two-dimensional Gas Chromatography coupled to time-of-flight mass spectrometry, was applied to screening the unknown organic pollutants in eight sampling sites from a typical reservoir in China. A total of 288 organic contaminants were identified, of which 9 were detected at 100% sites of study reservoir including aniline?1,4-dichlorobenzene?nitro-benzene?o-nitraphenol?2,4-dimethylphenol?2,4-dichlorinephenol?1,3-dimethylnaphthalene?1,4-dimethylnaphthalene?diphenylamine in 2017. And compare to the testing results in 2018, only 66 organic contaminants were identified, of which none compound was detected at 100% sites. The large number of organic pollutants decreased in study area, which from 2017 to 2018, due to the cage-cleanup-activities in this reservoir.

TU174

Are complex mixtures of xenobiotics common in groundwater?

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Although groundwater is considered relatively safe water resource being naturally protected by soil and rock layers, xenobiotics also occur in groundwater contaminated by agriculture, industrial sites, landfills, leaky sewers etc.. More than 200 man-made chemicals have been lately monitored in groundwater at almost 700 sites in the Czech Republic. The national groundwater monitoring network is set up the way that it does not monitor contaminated sites such as industrial areas, landfills etc. In spite of such setup, 120 chemicals were found in groundwater in various mixtures, up to 30 substances occurred in individual monitoring sites. In total 2715 samples were taken in years 2016-2017, thereof 528 (20%) samples were free of such compounds, 323 (12%) samples contained just one compound, 231 (8%) samples contained 2 compounds, 3 to 10 compounds were detected in 1255 (46%) samples and more than 11 up to 30 compounds were found in 378 (14%) samples. Prevailing contaminants were pesticides followed by VOCs (mainly chlorinated hydrocarbons) and PAHs. Also alkylphenols, pharmaceuticals, chelating agents, PCPs and benzotriazoles were detected in groundwater. The total concentration of xenobiotics in groundwater can exceed 100 and reach up to 551 μg/l. Monitoring results show that mixtures of various xenobiotics can be found in groundwater more often than one could suppose.

TU175

Identification of toxicants from a hydrocarbon contaminated sediment of a log pond

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Sediment from a log pond of plywood mill located in south Finland contained 15,000 to 50,000 mg/kg dw of C10–C40 hydrocarbons. It was unclear whether they originated from the hydraulic fluid of the log hoist or the wood extractives because biogenic organic compounds (BOCs) can interfere with chemical analysis of total petroleum hydrocarbons (TPH) by being extracted along with anthropogenic oil compounds. For risk assessment, additional information was needed on the nature of the hydrocarbons in the contaminated area, as TPH concentration often does not correlate with the results of toxicity tests. In the present study, methods of effect-directed analysis (EDA) were used for the identification of toxicants. The toxic potential was investigated using a biotest battery that included two in vivo acute toxicity tests, an estrogenicity test, and a genotoxicity test. A preparative fractionation of samples enhanced the potential to identify the key toxicants in BOCs and petroleum hydrocarbons. A combination of fractionation, biotesting and chemical analyses revealed that the key toxicant of log pond sediment was retene, a dialkyl-substituted phenanthrene derived from wood resin acids. Additionally, the most toxic fraction included three other wood-originated diterpenic compounds. Typical wood extractives such as sesquiterpenes and odd-carbon number alkanes in the range C21–C33 were identified in the fraction, which showed minor genotoxic potency. The most polar fraction contained triterpenes and showed estrogenic activity. No evidence for the presence of hydraulic fluid in sediment was found. The present study also indicated that in cases where the organic matter content of sediment or soil is high, using the results of standard mineral oil analysis in risk management can lead to incorrect actions, because standard methods do not differentiate petroleum hydrocarbons from naturally occurring hydrocarbons. However, sometimes environmental effects of BOCs can be larger than the impacts of petrogenic compounds if these compounds are concentrated at a particular location due to human activities. The present study showed that EDA can be applied in the identification of responsible toxicants to samples that are contaminated with different kinds of hydrocarbon.

TU176

How to measure time-weighted average concentrations with Diffusive Gradients in Thin films (DGT)?

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Passive sampling can be a cost effective analytical approach to provide time-weighted average (TWA) concentrations of pollutants in natural waters. DGT differs from other passive samplers by incorporating a diffusion disc in between the accumulation element (binding phase) and the sample [1]. The DGT labile concentration is defined as $c_{DGT}= n_M$ [delta]^g / (D_M A t), where n_M is the accumulation, D_M the metal diffusion coefficient, [delta]^g the total thickness of the diffusive gel, filter and diffusive boundary layer, A the area of the device and t the deployment time. Solutions of Ni, Cd or NiNTA were used to compare c_{DGT} with the average total metal concentration for different deployment times and metal concentrations.When the accumulation in simple metal solutions approaches perfect sink conditions, c_{DGT} provides a good estimation of the TWA metal concentration. Transient effects following each concentration jump are mainly dependent on the pulse time as it will be presented.In presence of ligands, it is shown that c_{DGT} depends on the speciation, the mobility and the lability degree, [xi], of the complexes at each time interval [2]. Since [xi]≤ 1 and usually the diffusion coefficients of the complexes are smaller than that of the metal, c_{DGT} can underestimate the TWA total metal concentration as seen in many cases[3]. For only one strong complex and excess of ligand conditions, the free metal is negligible and the lability degree becomes independent of the time interval. Then, c_{DGT} reduces to $c_{DGT}(t)= D_{ML}$ [xi] $c_{T,M,Av}$/ D_M. Thus, a simple factor can allow c_{DGT} to improve the estimation of the TWA metal concentration in presence of a dominant complex as seen in the NiNTA system. [1] Davison, W.; Zhang, H. 2012. *Progress in understanding the use of diffusive gradients in thin films (DGT) – back to basics.* Environ Chem 9: 1-13. [2] Altier, A.; Jimenez-Piedrahita, M.; Uribe, R.; Rey-Castro, C.; Cecilia, J.; Galceran, J.; Puy, J. *Effects of a mixture of ligands on metal accumulation in diffusive gradients in thin films (DGT).* Environ. Chem. 2018, 15 (3), 183-193 [3] Menegario, A. A.; Yabuki, L. N. M.; Luko, K. S.; Williams, P. N.; Blackburn, D. M. 2017. *Use of diffusive gradient in thin films for in situ measurements: A review on the progress in chemical fractionation, speciation and bioavailability of metals in waters.* Anal. Chim. Acta 983: 54-66.
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TU177

Development of Stainless Steel Filter as a Novel Passive Sampler for Chlorinated Paraffins in Waters

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Passive sampling has many advantages over active methods: providing time-weighted average (TWA) data, saving time and cost and can yield highly spatially resolved data. Of many passive samplers, the diffusion-based samplers have many advantages: such as the well-controlled sampling rate and a potential surrogate method for indicating mobility and bioavailability. The stainless steel filter (SSF) is used for solvent filtration before HPLC pumps, it has a well-defined wall thickness and pore size and it is inert to POPs. Therefore, we hypothesize that it might be possible to use it as a kinetic passive sampler by filling with the suitable sorbent. Chlorinated paraffins (CPs) is a mixture of polychlorinated n-alkanes of varying chain length (C10-C30) and degrees of chlorination (40 to 70% by weight). The CPs are generally grouped as short-chain (SCCPs, C10-13), medium-chain (MCCPs, C14-17) and long-chain (LCCPs, ≥ C18). Analysing CPs in the environmental samples is challenging, a cutting-edge analytical method for determining CPs has been developed and validated recently. The combination of this updated CPs analytical method with the kinetic passive sampling technique for in situ sampling of SCCPs, MCCPs, and LCCPs in the aquatic environment is of great interest. Herein, we will report the development of this SSF passive sampler for CPs in waters. We have tested that XAD-2 could be the suitable sorbent and further testings such as the time dependence, diffusion coefficient and effects of some key water chemistry factors are undergoing and the results will be presented at the conference.

TU178

Passive sampling as a tool for determining total chemical discharge in waste waters

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Currently industrial operators monitor their discharges mainly through analyzing the concentrations of substances in grab samples. However, if the emissions vary with time, the results from grab samples may give misleading information on prevailing chemical concentrations and emissions. Therefore, to achieve representative results through grab sampling, timing of the sampling is essential. Increasing the representativeness of the results might require more frequent sampling, resulting in increased analysis and sampling costs. With passive sampling, a more representative result on the prevalent concentration and occurrence of a large number of chemicals may be achieved. As the samplers are deployed for a length of time, the substances are enriched into the sampler material. This often enables the detection and quantification of smaller ambient concentrations than would be possible when using conventional grab sampling. Different matrices and varying conditions may affect the sampling procedure. The characteristics of waste waters vary dramatically depending on the field of industry. It may include e.g. a high concentration of suspended solids or solvents which attract the chemicals away from the dissolved form and make them unavailable for passive samplers. While grab samples are usually used in analyzing the total concentration of a chemical, passive samplers collect only the freely dissolved chemical fraction. The average dissolved concentration of the studied chemical in the matrix during the deployment can be calculated if the sampling rate is known. As operators are mainly required to monitor their total discharges, the results from passive samplers would either need to be converted into total concentrations by using substance- and matrix-specific conversion factors, or by limiting the use of passive samplers to only water soluble chemicals. The advantage of this technique is that a single chemical analysis may result in a temporally representative estimate of the ambient concentration of the studied chemicals. Thus, emission estimates would be more reliable than emission estimates based on few conventional grab samples. However, the results might not highlight e.g. transient high concentrations, which could have an impact in the receiving waste water treatment plant. To reach a comprehensive and reliable estimate on the emissions and range of chemical concentrations, it might be necessary to combine passive sampling with conventional grab sampling.

TU179

Analytical methodology for analysis of silicone materials in products

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With the January 2018 acceptance of a restriction of octamethycyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) by the European Union in wash-off personal care products and the future potential restrictions in leave-on personal care products and other consumer/professional products, the Silicone Industry has developed analytical methods for measurement of volatile methyl siloxanes (VMS) in silicone based materials. The methods currently available cover a range of silicone materials in the market place and take precautions to eliminate potential artefact formation of VMS during the analysis. This presentation details the following methods: 1) Quantification of residual amounts of VMS in fluid silicone polymers, which is applicable to silicone based sample matricesincluding hydroxyl-functional silicones ('siloxanols'), polydimethylsiloxane (PDMS) fluids, amino-functional silicones, polyether-grafted silicones (SiPE) and vinyl-functional siloxanes. 2) Quantification of residual amounts of cyclic volatile methyl siloxanes (cVMS) in silicone elastomers, which is broadly applicable to one-part and two-part pastes, sealants, heat cured elastomers, liquid silicone rubbers and room temperature vulcanized silicones in their cured and uncured states. 3) Determination of free cVMS in fully-formulated wash-off personal care products such as shampoos and conditioners. However, as the analysis of VMS is prone to false high findings depending on individual matrix interferences, users of these methods need to validate the methods in their own laboratory prior to analysis of unknown samples. Laboratories conducting the tests need a solid QA/QC program to ensure accurate results. Based on the current instrumentation recommendations, the methods are expected to work over a range of concentrations between 0.01%

and 0.5%.

TU180
Global monitoring of siloxanes in the environment: A commitment of industry in cooperation with governmental agencies and scientific community
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The Silicones industry, through the Global Silicones Council, committed significant resources to better understand the environmental presence and behavior of siloxanes used to make silicones. These siloxanes are known as octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6). Industry -initiated environmental monitoring programs, which are coordinated with government agencies, employ methodologies and quality control measures that can accurately measure low part per billion levels in the environment, and which may allow for a scientifically sound risk assessment. The following programs are described in detail and show the working relationship the GSC has developed with government agencies across the globe. 1) United States Environmental Protection Agency (EPA) D4 monitoring program. 2) European Union Restrictions monitoring program. 3) Voluntary long-term monitoring of D4, D5 and D6 in aquatic environments in North America, Europe, and Asia.

TU181
TOXmini® relevance and sensitivity assessment
M. LOT, F. Mounède, T. Merzi, P. Baldoni-Andrey, TOTAL SA
In a regulatory context of ever increasing environmental responsibility (OSPAR convention, BREF CWW), there is a need to have biomonitoring tools to evaluate waste water quality at any time. To date, and for several decades, the standard toxicity testing tool used for rapid analysis of waste water has been Microtox®. However, this tool is too complicated to be used on production sites (off-shore and on-shore) by operators without prior knowledge. A new tool has become available called TOXmini®. This one is an equivalent of Microtox® but is portative (lighter weight) and easier-to-use. Main difference between tools is the calculation of an inhibition rate as endpoint for TOXmini® instead of an EC_{50} for Microtox® which lead to an easier usage (no dilution series). TOXmini® was compared to Microtox® test (reference test) on typical substances and chemical additives found in the oil industry. The effect of the effluent temperature was also assessed to mimic the same conditions as we can find in a production site (high and low temperature). Results show similar results between the two tools and no temperature effect on bacteria viability was observed. Tests on other marine organisms (algae and rotifers) were also realised in order to compare results on organisms of different trophic levels. It appears that there is no general rules about organism sensitivity. Hence, results show that TOXmini® may be used as a biomonitoring tool on production sites. However, in cases where bacteria would not be the most sensitive species, the use of this tool will not be self-sufficient and would require to be combined with additional bioassays.

TU182
USE OF EFFECT-BASED METHODS FOR WATER QUALITY ASSESSMENT OF TIBER RIVER IN ROME
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The Tiber is the third-longest river in Italy and the main watercourse in Rome. As such, there has always been a historical and social relevance to the economic activities emerged along its banks. Indeed, the Tiber's river basin has been affected by different kinds of human pressure involving a number of potential sources of pollution, including pesticides, urban and industrial wastewater discharges, waste dumps and livestock waste products. It can be thus assumed the existence of thousands of chemical pollutants, also combined as mixtures, affecting its entire watercourse. Therefore, a monitoring strategy based on ecotoxicological bioassays has been elaborated through the analysis of organisms belonging to different trophic levels of a typical freshwater ecosystem, with a view to investigating the effects of such contaminants on aquatic biodiversity. A special focus has been placed on zebrafish at early life stages and their behaviour, as this model can provide fundamental data on the ongoing development of vertebrates exposed to toxic environmental samples. The goal of this study is to support the evaluation of the quality status of the urban section of the Tiber River in the city of Rome, which aims to implement the European legislation on waterbodies protection (Water Framework Directive). The results will help the scientific and administrative local communities in elaborating actions for reducing chemical pollution in one of the most populated areas of Italy and protecting both the ecosystem and human health.

TU183
Effect-directed monitoring tools as a toxicological fingerprint for ecological and human risk assessment of water bodies.
V. De Gussem, University of Utrecht / Environmental Sciences; L.M. Schuijt, Wageningen University; P. van den Brink, Wageningen University / Department of Aquatic Ecology and Water Quality Management; J. van Dijk, Copernicus Institute Utrecht University / Environmental Sciences; M. Jonker, N. Kramer, Utrecht University / Institute for Risk Assessment Sciences; A. van Wezel, University of Amsterdam IBED Institute
Classical analytical methods to monitor water quality have their limitations: i) not all chemicals of emerging concern can be measured at low enough concentrations in water, ii) they do not assess the presence and levels of all unknown chemicals (e.g. transformation products), and iii) they do not assess chemical mixture effects. For these reasons, the use of bioassays to assess the health risks associated with chemical contaminants in water has been promoted the past few years. We propose a battery of sensitive, 3R-based and practical bioassays, covering ecologically- and human-relevant toxicity pathways. These effect-directed monitoring (EDM) tools will assess the ecological and human health risks of chemicals in different types of water bodies, taking into account complex mixture of compounds. Endpoints of the bioassays were selected based on the modes of actions (MoA) or key events (KE) of human- or ecotoxically-relevant adverse outcome pathways (AOPs) reported for a compiled list of regulated environmental chemicals and prioritized chemicals of emerging concern (CECs). The selection of the bioassays was completed by technical and practical criteria, in order to facilitate the use of the EDM tools in the context of routine water quality assessment of surface water and drinking water. Using the assay battery, a toxicological "fingerprint" of a water sample can be obtained.

TU184
Mitochondrial toxicity of micropollutants in water samples measured by the oxygen consumption rate in cells
M.E. Müller, University of Tubingen; S. Vikstrom, Agilent Technologies; M. Koenig, R. Schlichting, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; C. Zarfl, University of Tuebingen / Center for Applied Geoscience; C. Zwiener, Environmental Analytical Chemistry, Center for Applied Geoscience, University of Tuebingen / Geosciences; B. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology
Many anthropogenic chemicals enter the aquatic environment every day and some of them have the potency to impair mitochondrial functions. Due to the crucial importance of mitochondria as energy factories in all eukaryotic organisms, these chemicals may pose a risk to organisms and ecosystems. However, little is known how environmental pollutants interact in mixtures. The quantification of the oxygen consumption rate (OCR) of cells using the Agilent Seahorse XF^{e}96 Analyzer has the potential to differentiate the various modes of action of mitochondrial toxicity of single compounds, including inhibition of the electron transport chain or the ATP-synthase and uncoupling of oxidative phosphorylation by destroying the electrochemical proton gradient. Thus, the aim of our study was to analyse the applicability to measure the cellular OCR with the Agilent Seahorse XF^{e}96 Analyzer to identify and quantify mitochondrial toxicants in environmental samples. Using reference chemicals of known mechanism and potency, three experimental designs for the OCR measurements on HepG2 cells were developed to quantify and differentiate the effects on the main components of the mitochondrial respiration. Enriched river water and 4 selected environmental pollutants that are potentially occurring in river water were then tested with these bioassay designs. The herbicide bromoxynil exhibited a strong uncoupling effect and did not affect the electron transport chain, whereas the fungicide azoxystrobin was a potent inhibitor of the electron transport chain. The biocide tributyltin inhibited both the electron transport chain and the ATP synthase, but its uncoupling effect could not be quantified because it was masked by the inhibitory effects. Extracts of river water samples were tested at relative enrichment factors (REF) of up to 200 in comparison to the original water samples without apparent cytotoxicity during the duration of the experiment. Uncoupling or inhibitory effects could be detected in various extracts of river water while extraction blanks showed no or very low effects. These effects were then validated in binary mixture experiments confirming concentration addition of the water extracts and single chemicals of known mode of actions. In conclusion, the assay setup developed for OCR measurements in cells is capable of determining the effect of environmental samples quantitatively and precisely and allows conclusions on the mechanism of mitochondrial toxicity.

TU185
Design of bioluminescent enzyme biotests for analysis of the complex mixtures
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Complex in composition vital components of the natural environment, such as soil or food of plant origin, are prone to the accumulation of potentially hazardous substances, so the analysis of their safety is one of the priority tasks of

environmental toxicology. Rapid and simple biotesting techniques are needed to control safety and minimize health risks to consumers. The bioluminescent enzyme inhibition-based assay has a good potency for complex mixtures analysis. The purpose of the study was to develop a scheme for design of new enzyme biotests, suitable for assessing the safety of natural and artificial mixtures of complex composition. To assess the safety of complex mixtures potentially containing polluting chemicals, we used a coupled enzyme system of luminous bacteria NAD(P)H:FMN-oxidoreductase+luciferase (R + L). The bioluminescent method is based on the detection of the inhibitory effect of the analyzed sample on enzymatic reactions by registering changes in the intensity of the emitted light. At the first stage, the sensitivity of enzymes to the action of potential pollutants of a complex environment was established. It was shown that the coupled enzyme system R + L is sensitive to a number of heavy metals and pesticides at the level of their maximum allowable content in food. At the next stage, the effect of the food samples as a complex media on the coupled enzyme system R + L activity in the absence of pollutants was assessed. When components of the control (unpolluted) food samples inhibited or stimulated the R + L activity we modified the procedure of the sample preparation to decrease the effect. At the final stage model experiments were conducted to assess the sensitivity of the coupled enzyme system R + L to a complex mixture of the analyzed sample of food and toxic substances. Thus, it has been established that the basic principles for designing bioluminescent enzymatic tests for analyzing heterogeneous media are 1) to ensure maximum sensitivity to potentially toxic substances and 2) to select the procedure of sample preparation that guarantee the minimal effect of unpolluted complex media on the coupled enzyme system R + L for correct interpretation of results. The reported study was funded by the Russian Foundation for Basic Research, the Government of Krasnoyarsk region and the Krasnoyarsk Regional Fund of Science according to the research project no. 18-44-242003.

TU186
Sensitivity of epigean versus hypogean crustaceans: ecotoxicity and online biomonitoring
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Groundwater represents an important reservoir as drinking water resource in Europe, prone to pollution by agricultural chemicals and nutrients. Risk assessment is being based on toxicity data of surface water species, mostly Daphnia spp. However, groundwater invertebrate species are facing completely different abiotic and biotic factors, life cycles and lifetime duration; hence extrapolation of toxicity data from Daphnia spp. to groundwater crustaceans might not be appropriate and underestimate the toxic potential of pollutants. Moreover, groundwater as safe drinking water resource needs to be protected continuously, e.g. by online biomonitoring. Within GROUNDCARE (BMBF 2015-2019) we study the acute and chronic effects of nutrients (nitrate) and toxins (Cu, BPA) on surface and subsurface crustaceans. We also develop groundwater crustaceans as new biomonitor species in the Multispecies Freshwater Biomonitor (MFB) for permanent online biomonitoring of drinking water intakes. First results indicate *Proasellus slavus* as most sensitive species compared to *Gammarus fossarum* and *Niphargopsis casparyi* regarding acute and chronic exposures to Cu and BPA. Moreover, among the microcrustaceans, *Eucyclops serrulatus* proved to be more sensitive than *Daphnia magna*. Groundwater macrocrustaceans proved to be at least as sensitive as *Daphnia magna,* easier to handle, and with longer stand-alone times in continuous longterm biomonitoring of water quality, thus allowing for recording integrated effects of chronic low-dose chemical mixtures.

TU187
In situ assays to assess the impact of wildfires on reservoirs
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Reservoirs are important and strategic water resources in Portugal, guaranteeing water for human supply and irrigation in a significant part of the territory. Hence, it is of fundamental importance to guarantee their water quality. Recently, wildfires have been pointed out as an important source of diffuse contamination to aquatic systems, affecting drastically their quality, namely through the production and subsequent exportation of deleterious pyrolytic substances, such as polycyclic aromatic hydrocarbons (PAHs), and through the input of metals associated to ash loads. Notwithstanding the recognition that wildfires can affect water quality, their potential toxic effects on aquatic ecosystems have been largely ignored. Hence, the main goal of this study was to assess the off-site effects of wildfire on a reservoir using *in situ* bioassays with key aquatic standard organisms. The bioassays were conducted in a recently burned area located in the Cabril catchment (central Portugal) just after the first post-fire rainfall events. Four sites were selected to perform the *in situ* assays: two in the main stream (ZD- downstream the burnt area and ZB – within the burnt area) and two in a tributary river (UB – within the burnt area and UU – upstream the burnt area (reference site)). The chemical composition of water was analysed, with specific focus on metal and PAHs. Three freshwater organisms, including the producer microalgae *Raphidocelis subcapitata*; the first consumer *Daphnia magna* and the benthic detritivore *Chironomus riparius*,were exposed in all four sites, using dedicated test chambers. For the microalgae the endpoint assessed was the growth rate inhibition, while for the other two species was the post exposure feeding inhibition. The chemical assessment proved the existing concern of wildfires impacts on aquatic systems, as a diffuse source of metals. The effects observed on the three species allowed discriminate unburnt and burnt sites. Thus, the *in situ* bioassays showed to be a suitable tool to assess the risks of wildfire to aquatic systems.

TU188
Biodegradation of Hydrocarbon Contents in a Crude-Oil Polluted Soil using Peroxidase from Fungal Di-Cultures (Rhizopus and Saccharomyces spp.)
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This study was carried out to evaluate the biodegradation of hydrocarbons in a crude oil polluted soil using peroxidase isolated from *Saccharomyces* and *Rhizopus* species. Crude oil polluted soil samples were collected from Bonny loading jetty site at Onne. Physicochemical analysis of the soil samples revealed that it was acidic (pH 5.02) with conductivity of 723. Dissolved mineral contents of the polluted soil revealed the presence of Cl, $P0_3$, Mg, K, Ca, $H(CO_3)_2$. Heavy metals identified in the polluted soil included Fe, Cd, As and Pb, while Cd, and Hg with higher values compared to the unpolluted soil. Hydrocarbon degrading peroxidase was produced from the fungal di-cultures isolated from the polluted soil using standard microbiological and biochemical techniques. Fourteen days pilot study carried out on the isolated fungi optimized for peroxidase production showed day eight (8) as the day of maximum enzyme production in the fermentation media. Optimum operational conditions for enzyme substrate reaction for peroxidase activity from the fungi were evaluated and observed at pH (4.5) and Temperature (50ºC). The degradation was carried out and evaluated using optimized gravimetric techniques. Peroxidase incubated with the crude oil in a basal mineral medium showed strength of utilization of the carbon catenation chains in all the optimized parameters. Peroxidase from the di-cultures showed optimal degradative ability at acidic ph range with the peak at 5.5 with the residual oil given as 15-20% v/v. Evaluation of biodegradation by the peroxidase showed that the total weight of degraded oil was 4.02g. The weight of oil degraded increased with increase in enzyme concentration. This study has shown that peroxidase has a great potential in the biodegradation of hydrocarbons present in crude oil polluted soil and is of high eco-toxicological relevance as regards to environmental remediationl. Keyword: Biodegradation, peroxidase, *Rhizopus* sp. *Saccaromyces* sp.

TU189
Effluent biodegradatibility evaluation using Whole Effluent Assessment
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In its Risk-Based Approach (RBA) for the management of Produced Water discharges from offshore installations1, the Oslo and Paris Commission (OSPAR) Recommendation 2012/5 advocates the Whole Effluent Assessment (WEA) approach. WEA approach increases the understanding of the combined effects in complex mixtures like effluents. The advantage of WEA approach compared to WET approach (toxicity only) is that WEA approach gives more information about the future of the effluent with the characterization of P (Persistency) and B (Bioaccumulation) criteria. A WEA practical guidance2 document from OSPAR Commission indicates some available persistency tests but without giving any information about their interpretation. Moreover, the review of the persistency test highlights the inconvenience that they were developed to assess the biodegradability of mono substances and not complex mixtures such as produced waters. Hence, there is a need to identify the most appropriate methodologies (tests) to evaluate the biodegradation of produced waters taking into consideration the specificities of substances included inside (naturally occurring substances and additives).

TU190
Environmental compatibility of soils debris conditioned with foaming agents containing sodium lauryl ether sulphate in a tunnelling scenario
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science and environmental technologies; A. Finizio, University Milano - Bicocca / Department of Earth and Environmental Sciences; L. Patrolecco, Water Research Institute-National Research Council / Water Research Institute

The mechanized tunnelling industry by EPB-TBMs (Earth Pressure Balance-Tunnel Boring Machines) needs a wide use of foaming agents and polymers as lubricating products for soil conditioning. The anionic surfactant sodium lauryl ether sulphate (SLES) is the main components of foaming commercial products. Soil debris from excavation processes contain residual concentrations of SLES and their potential re-use for environmental purposes depends on its persistence that is influenced by site-specific conditions. The objective of this study was to evaluate the overall environmental compatibility of different soils from real excavation sites conditioned with foaming products containing SLES. For this purpose, three soils in different lithologies (S1 clay; S2 sandy-clay; S3 clay) were conditioned with one common commercial foaming agent (F, containing 5-10% SLES) in presence or absence of a polymer (P, containing 25-50% SLES); this latter is used in some excavation conditions to increase foam persistence. Several microcosms were set-up mixing 1,5 kg of each soil with water, foam and/or polymer and then incubated at room temperature for 28 days. Control microcosms consisting of un-conditioned soil were also considered. At selected times (0,7,14,21,28 days), soil sub-samples were collected from each microcosm to analyse SLES concentration and to produce aqueous elutriates on which different ecotoxicological tests were performed. Terrestrial and aquatic test organisms were considered: the luminescent bacteria *Vibrio fischeri*, the plant *Lepidium sativum*, the worm *Eisenia foetida*and the ostracod *Heterocypris incongruens*. Moreover, SLES residual concentrations were determined over time both in soil than in elutriate samples with Methylene Blue Active Substances (MBAS) spectrophotometric method, preceded by Pressurized Liquid Extraction(PLE) of SLES in the case of soil matrix. The results showed an initial (t=0d) SLES concentration in the different conditioned soils ranging from 54 to 105 mg/kg, with the higher value detected in the soils (S1 and S3) conditioned with both F and P. The overall ecotoxicity tests showed that already at the starting time SLES concentration in soils and elutriates did not exert any toxicity effect on the organisms tested. This study highlights the important role of the ecotoxicological approach in order to assess if soil debris can or not pose a potential risk for the terrestrial and water compartments in order to use this soil as a by-product.

TU191

Characterization of fine (PM2,5) and ultrafine (PM<0,3) atmospheric particles under industrial or traffic influence in the North of France

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Airborne particulate matter (PM) is a complex mixture of particles from different origins. Compositions of PM strongly depend on the mechanism of formation, meteorology and mainly the nature of their emission sources. These latter are numerous, can be natural or anthropogenic and could be divided in stationnary (industrial sites, house heating …) or mobile sources (road traffic). As a result, the health impact of the atmospheric particles, especially on repiratory system, can change according to their origins. In this context, the objective of this work was to study the variation of composition of fine and ultrafine PM under industrial and road traffic influence before studying their impact on human epithelium. PM were collected at two different sites. First, Grande-Synthe, located on the French North-Sea coast. The sampling point was placed under the direct influence of Dunkerque port-industrial area. Secondly, in Roubaix, a city included in Lille metropolis, on a site located close to a high traffic road. PM were sampled from March to end of May 2017. Particles on plates and back-up filters collected with sierra 235 high volume cascade impactor were pooled to obtain average samples of fine and ultrafine particles. The PM were analysed for water-soluble ions, trace and major elements, n-alkanes and PAHs content. In addition, morphological characterization were realized by SEM-EDX. Average industrial and urban $PM_{2.5}$ concentration wer close with respectively 11.6 and 10.8 µg/m^3. Industrial samples show higher concentrations in SO_4^{2-} while NO^{3-} is the major soluble compound in urban PM. These compounds are linked with the sulfure emissions from industrial sintering plants and NOx emissions from road traffic. Regarding trace elements, Roubaix PM present high levels of Ba, Cu, Mo and Sn which are known tracers for vehicle metallic parts attrition. On the opposite, under industrial influence, Fe, Mn, Ni, V and Zn are the major elements, coming from the metallurgic plants in Dunkerque industrial area and heavy fuel oil combustion. PAH concentrations are two to sixteen times higher in industrial PM. If pyrogenic compounds are predominant on both site, they mainly come from vehicles for urban PM whereas they suggest coal combustion (coke oven) in industrial ones. In conclusion, particles sampled are clearly specific from industrial and traffic influence. So it will be relevant to study their respiratory impact of the PM and try to discriminate their toxicity.

TU192

Environmental leaching of steel shots

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EU commission has forbidden the use of lead in many commercial products. Also the use of lead shots in Ramsar wetlands and shooting ranges are under debate. The steel shots are very likely to replace the lead shots in future. Steel shots are usually made of recycled metal scarps and will also leach in acid wetlands and shooting ranges. Many times, shooting ranges in Nordics are in mineral soil areas where also groundwater is formed. The steel shot leaching affects to environmental fate of the alloy metals. The steel shot leaching was studied with two different leaching test methods: batch testing according to EN-SFS 12457-2 and alternating freezing-melting cycles in laboratory. Both tests were conducted in liquid solid -ratio 10. The test materials were mineral soil (as in groundwater areas) and wetland peats. Steel shots (Saga® Eurotrap steel) was added to both soil types as much as to cover approximately 20 years of steel shot shooting in common Finnish range. Among other rarer metals iron, chromium and nickel were leached in both test types and soils. In batch test metals were more readily leached than in freezing-melting cycles tests. The test method used affected to the results. The freezing-melting cycle test represents better the natural leaching of materials in the environment than the standard batch test. The steel shots were more readily leached in wetlands than in mineral soils resulting in higher concentrations for many metals. Also, different leaching pattern were observed for the soil types. In mineral soil the leaching behavior of metals was steadier than in wetland samples where the alternation in the metal concentration levels was common.

TU193

Industrial scale destruction of old chemical ammunition of the Great War on the western Front. Hundred-year-old forgotten contaminations?

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During WWI, ammunition had been used on an unprecedented scale. When hostilities ceased, huge ammunition dumps remained. Unexplosed ordnances were collected on former battlefields too. There was an urgent need to dispose million tons of hazardous ammunition. New methods for safely breaking down chemical shells were then developed.. After defusing, the rounds were emptied by perforation when the toxic contents were liquid, by steam or hot-water washing-out for TNT explosive shells and by open-burning for both chemical and explosive shells. These processes caused severe top soil contaminations especially on former burning-grounds of chemical shell. Recent research has been conducted on 9 burning-grounds in France and Belgium. To this day, no vegetation grows at some locations due to extreme high grade of heavy metal (Zn, Pb, Cd,…), ranging for Zn from 10 to 100 g/kg, chlorinated and /or brominated dioxin & furan (1 000 to 4 000 000 ng/kg), and arsenic (2 to 110 g/kg) when arsenical "*Blue Cross*" shells loaded with the sternutators diphenylchlorarsine and diphenylcyanoarsine were open-burned. Inorganic arsenical compounds are associated with organic by-products produced by the oxidation (diphenylarsinic acid) or thermal decomposition (triphenylarsine, As-PAH like phenylarsafluoren) of CLARKs. Brominated dioxin, energetic compounds (TNT, nitronaphtalin) and thianes (impurities of yperit) have been measured too. High resolution geomagnetic measurements had been conducted on a site where 1,5 million old chemical shells have been destroyed during the interwar period near Verdun, leading to severe top-soil contamination, crops and meat destructions and the sequestration of the ground. Open-detonation pits and systems of burning trenches have been recognized and carefully probed. Exposure to site-specific contaminants through the consumption of foodstuffs produced locally on the considered site was unlikely to be a health concern. However, as for inorganic arsenic, given the presence of highly contaminated zones, it was suggested that cereals should not be grown on certain spots. Spincourt is an example of the ignorance of soil contamination left behind by WWI in land-use planning. Further research is needed to assess these forgotten contaminations and sites and theirs related environmental risks.

TU194

Mixture of ligands influence on metal accumulation in Diffusive Gradients in Thin films (DGT). The interplay of labilities from concurrent complexes.

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The interactions between a trace metal and ligands present in natural waters largely determine this metal availability to biota and plants. Natural waters contain mixtures of metal cations and ligands. After many efforts devoted to the study of simple systems with only one ligand [1-2], it is timely to enquiry whether the mixture entails specific non-additive effects, i.e. to what extent is it possible to predict the collective behaviour of the mixture from the values of the lability degrees of each single ligand system (SLS). Thus, a series of experiments with DGT (Diffusion Gradients in Thin films) devices [3] were carried out to measure

Ni accumulations in laboratory solutions comprising either Nitrilotriacetic acid (NTA), Ethylenediamine (EN) or mixtures of both ligands. It will be shown that NiEN and NiNTA can become more labile and inert (respectively) in the mixture than in the corresponding SLS. This "mixture effect" arises when one of the ligands (NTA, forming strong and partially labile complexes) is present at non-excess conditions. As variations in the lability degrees of NiNTA and NiEN arising in the mixture tend to cancel out, the summation of partial fluxes calculated from lability degrees obtained in SLS yields a reasonable estimate of the DGT performance in the mixture [4]. Numerical simulation will be used to justify this mixture behaviour. References: 1 Mongin, S.; Uribe, R.; Puy, J.; Cecilia, J.; Galceran, J.; Zhang, H.; Davison, W. *Key role of the resin layer thickness in the lability of complexes measured by DGT.* Environ. Sci. Technol. 2011, 45 (11), 4869-4875. 2 Uribe, R.; Mongin, S.; Puy, J.; Cecilia, J.; Galceran, J.; Zhang, H.; Davison, W. *Contribution of partially labile complexes to the DGT metal flux.* Environ. Sci. Technol. 2011, 45 (12), 5317-5322. 3 Puy, J.; Galceran, J.; Rey-Castro, C. *Interpreting the DGT measurement: speciation and dynamics*. In *Diffusive Gradients in Thin-Films for environmental measurements*, Davison, W., Ed.; Cambridge University Press: Cambridge, 2016. 4. Altier, A.; Jimenez-Piedrahita, M.; Uribe, R.; Rey-Castro, C.; Cecilia, J.; Galceran, J.; Puy, J. *Effects of a mixture of ligands on metal accumulation in diffusive gradients in thin films (DGT)*. Environ. Chem. 2018, 15 (3), 183-193. *Acknowledgement* - The authors gratefully acknowledge support for this research from the Spanish Ministry MCIU/AEI (Project CTM2016-78798) and FEDER EU

TU195

Daily behavior and removal of Rare Earth Elements in the wastewater treatment plant of Grand Nancy (France)

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Rare Earth Elements (REEs) have wide and growing applications in high efficiency electronics and energy technologies. These anthropogenic uses already disrupted the biological cycle of REEs and led to enrichments of La, Ce, Sm, and Gd in waters. Some REEs contained in sewage are not removed by conventional Wastewater Treatment Plants (WWTPs), notably anthropogenic Gd, which is particularly stable due to its organic complex form[1]. Furthermore, the concentration of some REEs measured close to a WWTP effluent discharge can be high enough to elicit effects on aquatic systems[2]. The presence of REE in sewage may also affect some aspects of treatment efficiency within WWTPs, particularly on the microbiological part[3]. Knowledge of the REE mixtures, abundances and distribution in WWTP influent and effluents is therefore necessary to evaluate REE removal efficiency and possible REE-related risks to living organisms within the WWTP and in the receiving waters. Presence of REEs in the influent and effluent of Grand Nancy's WWTP (350,000 population equivalent) was investigated. The Grand Nancy sewage network is mixed. The WWTP is of the activated sludge type with primary settling and chemical phosphorus precipitation. Twenty-four hours sample campaigns were conducted for each week day, in the aim to determine the REEs distribution and fate of a "typical week". REEs concentrations were measured in the total fraction after mineralization and in the dissolved fraction (< 0.45 μm). Results show that there is a Gd positive anomaly in influent wastewater, as well as in treated wastewater. The highest Gd concentration signal in influent appears mostly in late afternoon. Light REEs (from La to Eu) are preferentially removed from the influent wastewater during the treatment. References: [1] Möller, P.; Paces, T.; Dulski, P.; Morteani, G. Anthropogenic Gd in surface water, drainage system, and the water supply of the city of Prague, Czech Republic. *Environ. Sci. Technol.* **2002**, *36*, 2387–2394. [2] González V, Vignati DAL, Pons M-N, Montarges-Pelletier E, Bojic C, Giamberini L. 2015. Lanthanide ecotoxicity: First attempt to measure environmental risk for aquatic organisms. Environmental Pollution. 199(0):139-147\n [3] Fujita Y, Barnes J, Eslamimanesh A, Lencka MM, Anderko A, Riman RE, Navrotsky A. 2015. Effects of simulated rare earth recycling wastewaters on biological nitrification. Environ Sci Technol.

TU196

Cocktail effect of nanoTiO2 mixtures with mercury to green alga Chlamydomonas reinhardtii

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In aquatic environment nanoparticles are present as complex mixtures with other contaminants which can result in synergism, aditivity or antagonism of their combined action. Despite the fact that the toxicity and environmental risks of engineered nanomaterials have received extensive attention in recent years, their interactions with other contaminants and the subsequent effects to aquatic microorganisms are to explore. In such a context, the present study aims to examine the interactions and effects of cocktails containing nanoTiO_2 and mercury compounds, as a representative of priority hazardous substance, to *Chlamydomonas reinhardtii*, as a model phytoplankton specie. It was hypothesized that due to the its high reactivity nanoTiO_2 will adsorb Hg compounds and thus decrease its bioavailability and effects to alga, and that the effect will be more pronounced for cocktails containing nanoparticles of smaller primary size. The influence of nanoTiO_2 of different primary sizes, 5 nm (anatase, A5), 15 nm (anatase, A15) and 20 nm (anatase/rutile AR20) on inorganic Hg (IHg) and monomethyl Hg (CH_3Hg) effects to algae was studied. The effect of Hg, nanoTiO_2 and their mixture on the algal growth, generation of oxidative damage and membrane damage were determined by flow cytometry. In parallel, the stability of nanoTiO_2 in terms of hydrodynamic size and surface charge as well as Hg adsorptive capacity of nanoTiO_2 were determined. Results showed that the increasing concentrations of nanoTiO_2 with different primary size lead to a decrease of Hg-induced effects, due to the adsorption of Hg to nanoTiO_2. NanoTiO_2 had much higher adsorption capability to IHg than CH_3Hg.The results highlighted the need for improved understanding of the interactions of complex environmental settings containing mixtures of nanomaterials and other contaminants, central for sustainable development of nanotechnology. **Key word:** nanoTiO_2, mercury, phytoplanton, toxicity

Advancing Chemical Substitution and Alternatives Assessment: Challenges, Opportunities, and Approaches (P)

TU197

A Safe by Design Nanobiostimulant Tested on Alfalfa (Medicago sativa) - Effectiveness, Mode of Action, and Social Acceptance

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Safe by Design (SbD) nano-agrochemicals promise higher efficiency than conventional pesticides, and therefore have a high potential in reducing the pollution penalty of these products. However, before they can be implemented, much has to be learned about the gain of efficiency compared to conventional products, the economical and environmental costs of a large-scale production, the willingness of farmers to adopt their practice, and the risk of directly applying such new types of yield enhancers on agricultural soil. Here, we present the results of laboratory scale experiments to develop a bioinspired nanobiostimulant and compare its effects with conventional pesticide ingredients on the agricultural legume alfalfa (lucerne, *Medicago sativa*). As a reference substance for conventional pesticides, the broadband fungicide tebuconazole was tested. Seed germination and infection tests, and plant growth tests as well as biochemical tests with pathogens were conducted. The nanobiostimulant was quantified by inductively coupled plasma – optical emission spectroscopy (ICP-OES), and its interactions with the plant were visualized in view of potential uptake and translocation using transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS). In addition, interviews were conducted in Switzerland to evaluate the needs and willingness of farmers to adopt new agricultural strategies. The performance of the nanobiostimulant was found to be up to twice as high as for the conventional product. The evaluation of the results of the interviews is in progress and will be presented in detail at the conference. The use of nanobiostimulants promises to strongly reduce the organic pesticide burden of people and the environment.

TU198

Certification of field-operating personnel involved in environmental measurement and sampling improves the quality of environmental data in Finland

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Representative environmental measurement and sampling are essential for reliable environmental research and monitoring, and high-quality nature inventories are elementary parts of well-prepared land-use planning. The results of field operations are used e.g. to monitor water and soil status. Based on the results of field work, big and economically significant decisions concerning e.g. waste water, soil refurbishment and land-use planning are often made. Error or negligence in field operations can easily be missed, but its effect may be great. The complexities of diverse natural environments can make truly representative sampling a tricky task, and incorrect sampling may greatly distort research findings. Sampling may therefore in many cases be a delicate process and the most demanding part of environmental measurements. This is mainly due to natural heterogeneity of the environment in space and time. In addition, the content of the field operations always depends on the nature and scope of the information that is needed. The competence and responsibility of the field operating personnel is therefore critical for representative environmental field operations. Field personnel need to have sufficient knowledge, skills and detailed information about the aim of the field work to assure the representative sampling, measurement or observations. The Finnish national environmental certification scheme for environmental sampling personnel is an independent system enabling environmental field operators to demonstrate their competence in different fields of environmental sampling. Certified field operators have systematic sampling, measurement and experience. In addition, they undertake to maintain and develop their skills and update their knowledge during the certification period. The Finnish national certification system is a widely acknowledged quality assurance procedure today. The field-work quality has improved both in governmental monitoring programs and in private companies. Education of field operators in Finland has improved significantly due to the certification system. More courses are available and the contents of the courses have improved. Certified field operators are also more motivated to take part in training courses regularly.

TU199

Development of sustainable surfactant-based formulations: studying the influence of ethylene oxide head groups on the toxicity of SLEnS-LAS micelles

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Different formulations of surfactants (SF) are commonly added as active ingredients in biocides, industrial processes, cleaning agents, personal care and household products. Anionic SF are easily produced with lower cost associated, resulting in higher consumption levels worldwide. When in mixture, anionic surfactants like ether sulfate based (SLE_nS) and linear alkylbenzene sulfonic acid (LAS) form mixed micelles, acting as reservoirs for the cleaning process. SLE_nS with higher number of ethylene oxide (EO) units in the head groups present lower critical micellar concentration,reducing the quantity of chemicals needed to form the micelles therefore resulting in economic and environmental advantages. Despite its wide use in commercial products, the impacts of these micelles and their EO units' variations on soils are not available so far. Following this context, in this work were carried out ecotoxicological tests to assess collembolans reproduction (*Folsomia candida*), seed germination and plant growth *(Avena sativa, Hordeum vulgare*, *Lactuca sativa*, *Lycopersicum esculentum*) with main goal to assess the influence of the number of EO units in the sublethal toxicity of seven variants of SLEnS-LAS micelles. All the variants were tested in concentrations ranging from 247.5 to 1247.5 μg SLE_nS kg^{-1} of $soil_{dw}$. The majority of the significant ($p< 0.05$) inhibitory effects on plants growth are observed at the highest concentrations of almost all the substances tested with no apparent correlation with the EO units. Regarding collembolans reproduction, the formulations with less EO units (0 to 11) significantly inhibited the average number of juveniles produced with observed effects at the lowest concentrations tested and resulted in EC_{50} values ranging from 924.18 to 1025.04 μg SLE_nS kg^{-1} of $soil_{dw}$ while no significant results were obtained for the micelles with higher number of EO units (50 and 30).The results of this work contribute to the confirmation that a different design of chemical compounds may result in different responses to terrestrial organisms of different trophic levels. In this way, this research proves that the upstream use of standardized ecotoxicological assays, prior to their manufacture and marketing, may support an efficient precautionary attitude concerning the rising demand for new and more effective surfactants formulations, promoting the search for sustainable alternatives without detracting the required functions.

TU200

Further examination of the Hansen Solubility Parameters for prediction of hazard and exposure properties

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Hansen Solubility Parameters (HSP) – parameters predicted based on the molecular structure of a chemical - were developed originally to give information about solubility parameters of solvents and assisting in the choice of the appropriate solvent. This study explores the further use of HSP to not only predict solubility parameters, but to give information on hazardous effects of solvents as well as exposure properties. Comparing these properties can assist in sustainable solvent choice. An initial investigation of the use of HSP for description of certain hazardous properties of substances such as bioaccumulation potential and toxicity to aquatic organisms has been undertaken. These parameters are of significant regulatory interest, for example, in the identification of substances with persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) properties. This work has been extended to examination of adsorption of substances to soil and sediment. The present work explores the predictability of these properties using HSP and discusses the successes and limitations identified, and potential for future development. Challenges in respect of QSAR are being identified, as the HSP are of clear value but do not often follow linear relationships with the desired end point.

TU201

Learning from the past to design better chemicals

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Green chemistry based approaches are focused on the maximizing the generation and use of substances and processes that have limited/no impact on humans and the environment. Among the 12 green chemistry principles, particularly principle 4 "Design benign chemicals" and principle 10 "design for degradation" are useful to prevent hazardous substances, such as those characterized by PBT properties (Persistent, Bioaccumulative, Toxic) from being developed and entering the environment, as well as to generate safer alternatives to existing hazardous chemicals. Modelling approaches based on Quantitative Structure-Activity Relationships (QSARs) rely on the assumption that biological activities/properties of chemicals are intrinsically dependent on the molecular structure. QSARs can be applied, also in combination with data mining (data exploration) techniques, for the rational design of chemicals before their synthesis (i.e. Safe by Design – SbD approach) or after, to detect safer alternatives to undesired chemicals. Endpoints like toxicities, physico-chemical properties as well as biotic and abiotic degradations can be predicted starting from models based on molecular descriptors of the chemical structure. These models are available from the literature, regulatory tools, or are developed ad hoc for a specific endpoint. In this poster, we show the different steps necessary for a correct development, validation and application of QSAR models taking into account regulatory requirements, as defined by OECD and ECHA. In addition, we provide examples of how these models in combination with multivariate explorative analysis (MVA), are applicable to distinguish between desirable/safe and undesirable/unsafe compounds on the basis of different endpoints such as toxicities/activities, bioaccumulation and biotransformation properties, and environmental degradations. These examples show how *in silico* based screening of chemicals, before and after the chemical synthesis, can provide concrete opportunities to identify benign chemicals and alternatives for undesired chemicals by maximizing the existing knowledge through lessons learnt from the past.

TU202

Risk profiler at work - comparing environmental risk profiles of insecticides

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The environmental risk assessment for authorisation of pesticides is targeted at individual compounds in individual uses and serves the purpose of limiting the risk from each single pesticide to an acceptable level. Additional concepts in risk assessment now also aim at classifying and comparing levels of risk between pesticides. One is the substitution concept according to Article 50 of the pesticide regulation (EC) 1107/2009. Comparison of risk levels is also required for identifying pesticides with a more environmentally favourable risk profile than others, or for risk benchmarking, where the risks arising from the use of one pesticide are set in relation to the risk levels of other pesticides authorised for similar uses. A major challenge when comparing environmental risks from pesticide use is the diversity of environmental compartments, organisms and endpoints to be considered if a holistic assessment is intended. Moreover, acceptability of risks may either be demonstrated using standardised data and procedures (so-called "tier 1") or might require a specifically refined assessment (higher tier). While tier-1 risk indicators for the same endpoints can be compared directly, this is not necessarily the case for the results of higher-tier assessments. In order to facilitate the comparative assessment of pesticides, the German UBA has developed an approach using tier-1 risk profiles. These profiles are generated with an Excel tool and consist of tier-1 risk indicators for all assessment areas based on a small set of basic data for the pesticides, in combination with automatically assigned exposure scenarios for the relevant target crops. By comparison of corresponding risk indicators, conclusions can be drawn on possible critical risk levels for each pesticide and the magnitude of risk differences between pesticides, respectively. The results of a case study comparing the risk profiles of several insecticides from different mode-of-action classes will be presented. The outcome is discussed with regard to (i) the possible added value of comparing risk profiles in pesticide risk assessments and (ii) the predictive power of standardised pesticide risk indicators (i.e. tier 1 versus higher tier).

TU203

Sustainability assessment implementing the Safe by Design concept for innovative nano-enabled products in the field of cultural heritage conservation

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Nanotechnology provides innovative and promising solutions for the conservation of cultural heritage, but the development and application of new nano-enabled products pose concerns regarding their human health and environmental risks. To address these issues, we propose a sustainability framework implementing the

Safe by Design concept to support product's developers in the early steps of product development, with the aim to provide safer nano-formulations for conservation, while retaining their functionality. In addition, this framework can support the assessment of sustainability of new products in comparison to their conventional chemical counterparts. The goal is to promote the selection and use of safer and more sustainable nano-based products, along their life cycle, in different conservation contexts. The application of the proposed framework is illustrated through a hypothetical case which provides a realistic example of the methodological steps to be followed, tailored and iterated along the decision-making process. The proposed framework should not be considered as restricted to cultural heritage science and to nano-based formulations but can be extended to the development of innovative chemical products across various application domains, regardless of whether they occur as individual substances or in mixtures.

TU204
The regulatory counterpart of Safe-by-Design: Regulatory Preparedness for nanomaterials and other innovations
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While industry is adopting the Safe by Design concept for developing innovative materials, technologies and products that meet requirements for the protection of human health and the environment, regulators for their part need of their own concept for staying up-to-date on emerging innovations and preparing to assess and ensure their safety. Such preparedness can both enhance the safety of innovative products entering the market and avoid unnecessary delays in market introduction. At the NanoReg2 Workshop on Regulatory Preparedness for Innovation in Nanotechnology (October 2017), Regulatory Preparedness was defined as the regulators' timely awareness of innovations and actions to check whether present legislation covers all safety aspects of each innovation, including initiating revision of the legislation as appropriate. Together, Regulatory Preparedness and Safe-by-Design constitute the NanoReg2 Safe Innovation Approach for developing innovative products based on nanotechnology. However, while the focus is on innovations in nanotechnology, the workshop outcomes are relevant for regulatory approaches to the safety of innovative technologies in general. The workshop participants (ca. 60 regulators, industry representatives and other stakeholders) presented views on and identified current practices in regulatory work on the safety of innovations, tools that are already in use or would be needed, and potential difficulties in implementing Regulatory Preparedness in the European Union. Regulatory Preparedness was seen to require from regulators a combination of continuous and interconnected anticipatory activities (e.g. horizon scanning), together with appropriate resources. Since industry has a corresponding need to be aware of regulatory requirements concerning their innovations and how these requirements are likely develop, communication between regulators and innovators is of key importance. However, mutual trust must be ensured for the useful exchange of information that can involve valuable intellectual property.

Life Cycle Impact Assessment (P)

TU205
Development of Air Pollution Characterization Factors for African countries
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The 2017 United Nations previsions predict that the African population composed of 1.2 billion people nowadays might reach 2.5 billion by 2050, with more than 400 million people, Nigeria is projected to be the 3rd world population by 2050. From an economic perspective, according to a PWC report published in 2017, three African countries will be among the thirty strongest economies in 2050: Nigeria(14th), Egypt (15th) and South Africa (27th). This population and economic growth will for sure have an impact on the environment in Africa, the continent is already experiencing several environmental issues including Air Pollution, Water scarcity, Human Toxicity or Land Use degradation as highlighted by the 2013 UNEP report: African Environment Outlook (AEO-3). Additionally, according to the WHO (2018), more than 500,000 deaths occurred in Africa due to ambient air pollution in 2016. From the viewpoint of LCA, the number of groups focusing on LCA in Africa is still very limited compared to other regions of the world (Bjorn et al 2013). Even if regionalization has been getting a trend in LCA, nothing has been really made for the African continent. Specific LCI databases and LCIA methods have not been developed yet. For example, for the damage of air pollution, the current global LCIA methods RECIPE2016 and LIME3 divide the continent and its 54 countries into only respectively six and two parts. Therefore, this study aims at bridging the research gaps by providing specific characterization factors for African countries concerning Air Pollution. Our goal is also to evaluate the future environmental burden by establishing some projections for the characterization factors. In order to develop these factors expressed in DALYs per unit of pollutant, four relevant substances were chosen in accordance with the previous studies: Primary PM2.5, NOx, SO2 and NH3. Using a global Chemical Transport Model (CTM), the increase of concentration of PM2.5 in each country/region was evaluated following a rise in the emissions of precursors. Later, the latest global health estimates provided by the WHO were used in order to evaluate the possible effects and damages on the African population.

TU206
Habitat quality across the Scandinavian boreal forest from remote sensing and citizen science observations
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The increasing human population and associated growing pressures on ecosystems have already resulted in a significant loss of natural habitat and biodiversity. One of the strategic goals of the Aichi biodiversity targets is to halt this development by decreasing the human impacts. Therefore, biodiversity monitoring and decision-making processes have to be more effective. A tool for assessing environmental impacts is Life Cycle Assessment (LCA). In LCA, impacts on ecosystem quality are calculated by linking declining habitats to species loss following an ecological concept called species-area relationship. However, this does not account for variability in species richness within regions or within land use types. The massive amount of species observations by members of the general public, i.e. citizen science observations, may help to tackle those shortcomings by improving our knowledge on the distribution of biodiversity, by filling data gaps and by reducing time-consuming field campaigns. Hence, a potentially efficient way of advancing in biodiversity conservation is the implementation of citizen science observations into biodiversity monitoring. In this case study, we want to predict habitat quality across the Scandinavian boreal forest by combining citizen science observations of selected forest bird taxa and spectral information from satellite imagery as well as other remote sensing derived variables. Taxa are selected based on ecology and habitat requirements. We aim to illustrate the importance of citizen science efforts for decision-making processes. This feedback to citizens is likely to improve data collection and thereby prediction accuracy in the near future. The developed framework is expected to be applicable in other regions and ecosystems. Furthermore, the products can be integrated into decision-making processes and environmental impact assessments, such as (LCA).

TU207
Comparison of USEtox and an H-based approach for eco-toxicity based ranking of chemical emissions from an industrial cluster
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Impact assessment methods developed in an LCA context have shown useful as tools for ranking and priority setting also in other contexts, such as for national chemicals release inventories. The study presented here is a case study to prioritise eco-toxic risks based on emissions from a group of industries (a "cluster") in Stenungsund in Western Sweden. In additon to applying USEtox™ ecotoxicity characterization factors (v 2.0) for this purpose, a novel approach based on H-phrases for ecotoxicity was developed as a rapid way to include all relevant substances, inspired by the approach by which ProScale™ was designed for human toxicity potential assessments. The total amount of each substance emitted during 2000-2016 (in kg) has been calculated for the cluster, then multiplied with USEtox CFs to obtain the potential toxicity of the different substances. Some of the emitted substances were not specified, in these cases a CF was chosen from a representative chemical of the group. H-phrases were gathered from PubChem and formulated into two different ranking methods. The ranking methods uses environmental H-phrases (H4-phrases) to evaluate the toxicity of substances. The first method (version 1) uses the different variations of H-phrases within the list of substances to create eight different ranking levels. The second method (version 2) only uses the most severe H-phrase in its ranking and consists of seven levels. The two different H-phrase ranking methods was also compared to the CFs of substances emitted to water. For both the H-phrase approach and USEtox, the metal pollution turned out to be responsible for the highest toxic potential along with some of the non-specified chemicals. Halogenated organic compounds (AOX), chlorides and non-methane-volatile-organic-compounds (NMVOC) also have a high toxic potential. This might be because of the high toxicity of the representing chemicals for the groups. Chemicals with high CFs were chosen to avoid underestimation of the toxicity of these group, but this could possibly result in an unrealistically high toxic potential. In the comparison of the CFs of substances emitted to water and the H-phrase rankings there was much variation. This is realistic since the variables have different structure, CFs contains a fate-factor while H-phrases do not. To continue developing the H-phrase approach, a calibration with effect-based hazard factors, such as EC50 or HC50, would be relevant

TU208
Regionalized quantification of the loss of aquatic insect species due to river

transformation into reservoir
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Freshwater biodiversity, is critically threatened by flow alteration and habitat degradation, which are commonly associated with hydropower. The first and only attempt to assess potential impacts from water stream use and management in life cycle assessment (LCA) on aquatic biodiversity, is an unpublished exploratory work focusing solely on fish species. As a consequence, this impact pathway has yet to be characterized in LCA. This project aims to develop LCA characterization model and factors to assess the impacts of hydropower production on freshwater biodiversity, focusing on aquatic insects and based on strong empirical natural science. In this paper, we focused on the occupation impact pathway (similar to a “land occupation” impact pathway which is leading to impacts of transforming and occupying the original bed of a river that was transformed into a reservoir following impoundment) and developped a characterization model and factors that quantifies the impacts of hydropower occupation within LCA (i.e., the transformation of a river bed into a reservoir due to an impoundment). We used two datasets from the US Environmental Protection Agency, one pertaining to lakes and reservoirs and the other, to rivers. We calculated a mean PDF for the overall US and accounted for regionalization by calculating a specific PDF for nine ecoregions in the US. We also calculated an impact score by multiplying the characterization factor (PDF) with the mean surface of river occupied (i.e., original riverbed) and assuming a lifetime of 100 years for each of the reservoirs. Preliminary results showed an overall loss of aquatic insect species across the US, as well as in five out of nine ecoregions. Two ecoregions showed a marginally significant loss of species, one ecoregion showed a non-significant gain of species and one last ecoregion showed a significant gain of species. Overall, our results show that the transformation of a river into a reservoir does lead to a loss of aquatic insect species. Regionalization also seems to be playing a relatively important role considering the different levels of significance regarding loss, and gain, of species. Further analyses still needs to be conducted to identify and understand what are the underlying mechanisms that rule the similarities/differences among ecoregions. Eventually, these results could help better our understanding of the environmental cost associated with the use of hydropower.

TU209
Water footprint and regionalization: the case study of Walloon corn
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Corn is an important cereal with a lot of applications in the feed and food industries (e.g. starch production). To evaluate properly the environmental impact of its applications, for example in the growing context of biobased products, a better understanding of the impact of its production is needed, using Life Cycle Analysis (LCA). The studied system is the production of corn in Wallonia (South of Belgium), whose the primary data are taken from Van Stappen et al (2018. Sensitive parameters in local agricultural life cycle assessments: the illustrative case of cereal production in Wallonia, Belgium. Int J Life Cycle Assess 23 2: 225–250). The functional unit is 1 hectare of corn crop in Wallonia. The LCI data are based on actual agricultural practices recorded in farms’ accounting data. The system has been modelled in GaBi 8 using GaBi datasets. The ILCD recommended method has been used. To have a better understanding of water depletion, some other methodologies have been tested for this specific impact categorie such as ReCiPe and the AWARE model. The part of water resources depletion using ILCD recommended method that is not due to the fertilizers (20 %) production is mostly related to the electricity used for maize drying and storage (70 %), especially associated with nuclear electricity (cooling needs). This is not the case if another method is used for assess the water depletion. When AWARE method is used, most of the impact is coming from fertilizers production and from mechanization (due to the use of on unspecified flow for water consumption), whereas, using ReCiPe, most of the impact is from fertilizers production only. A study of the water origin has been realized using AWARE and ILCD recommended method and underlines the large variety of results. The share of nuclear electricity in water depletion is really high when using ILCD recommended method, therefore special attention has been put on this process.: we have compared the GaBi dataset with the Ecoinvent dataset. For the Ecoinvent dataset the calculations have been realized in GaBi but also in Simapro. This comparison is realized for the three methods investigated and underlines large differences between softwares. Therefore, this contribution allows to underline the difference between methods for water depletion calculation but also the differences induced by regionalized or not regionalized datasets. Finally, it highlights the problem of results that are dependant of the software.

TU210
Green water scarcity under climate change: a life cycle impact assessment method applied to Portugal
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Climate change is one of the greatest challenges today. The debate now is not about the human contribution to climate change, but how to deal with the impacts of future climate change, namely regardingthe risks of water stress and fresh water scarcity, with consequences on food production, energy and other economic sectors, human health and ecosystems. The impacts related with water use, and mainly the impact of green water flows, are particularly relevant for agricultural and agro-forestry based products, depending on local land-use and land cover changes. Green water flows refer to the portions of green water (from rain) used by vegetation that is evaporated or transpired. This is an on-going research issue in Life Cycle Impact Assessment (LCIA), since the environmental mechanisms of green water consumption are not yet well understood. This study is devoted to develop green water scarcity characterisation factors (CFs) at mid-point level for Portugal, considering a high spatial resolution (9 x 9 km) both for reference climate (period 1986-2005) and future climate (period 2046 to 2065). These CFs were calculated considering two different interfaces: (1) interface green water – soil (ws), in which the relation between the changes on green water flows and the surface blue water production is considered, and; (2) green water – atmosphere (wa) interface, in which the relation between the evapotranspiration recycled to the atmosphere and the precipitation levels at the same catchment level is considered. Considering the reference climate conditions, it was noticed that some regions of Portugal are currently suffering of pressure on green water resources (mainly in Central littoral and coastal areas). Under future climate conditions, a global increase of CF_{wa} at the country level was observed. For the ws interface, the CF_{ws} mainly increases in the South coastal and Central interior areas, whereas in coastal areas (Northern and Centre) the CF_{ws} decreases comparatively to those for the reference climate. This CF_{ws} decrease means that the availability of green water required to produce blue water is ensured. These CFs contribute to the operationalization of the green water scarcity footprint methodology for decision support of agricultural and agro-forestry producers and policy makers. Furthermore, the approach used in this study can be, with a reasonable amount of effort, fully applied to other countries.

TU211
Quantitative Evaluation of Rice Production Considering the Impact of Light Pollution
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In recent years, it became clear that the brightness of artificial light at night has been increasing year by year, and its scale is expanding. According to Science Advances, it is reported that from 2012 to 2016, the artificially illuminated outdoor area increases by 2.2% per year and the annual radioactivity total radiance increases by 1.8%. Light pollution is described as the situation in which these lights are obstructing or having damages through their leaks, a common example is the outdoor lighting. Light pollution has various effects on human activities, wild animals, plants, agricultural crops and livestock. Rice is also one of the crops affected by light. Rice is a short day plant where flower buds begin to form when the light exposure becomes longer than the critical dark period, which is the minimum requirement time needed for flowering. However, today road and sign lightings are increasing with the countryside urbanization. Therefore, the influence of light pollution on rice is expected to increase due to the increase in artificial light at night. Although the influence for each region and each type of rice must be considered, the evaluation in Japan as a whole has not been done. Growth of rice is hindered by night lighting, and light pollution has become a problem as a type of pollution causing heading delay. There are no examples of investigations on light hazards in LCA research. There is also no light pollution in the item of impact category of the LIME method. In this research, we aim to conduct a quantitative evaluation of rice production considering the impact of light pollution, using existing research related to light pollution.

TU212
Health impact evaluation of PM 2.5 in two Korean cities by using LCIA method
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This study evaluated health impacts of PM2.5 in two Korean cities, J and I by using life cycle impact assessment method. Site-specific intake fractions were obtained by multiplying the human inhalation exposure factor and fate factor. Effect factor was obtained by multiplying severity factor and dose-response factor. Finally, the characterization factor ($DALY/kg_{emitted}$) was calculated by using the intake fraction and the effect factor. Emission inventory (year 2015) of PM2.5,

NO_x, SO_x, and NH_3 was also used to find relative PM2.5 contribution of emission source to human health. Human health impact of J and I cities due to inhalation of PM2.5 was quantified as 2,606 and 683 DALY, respectively. The prominent PM2.5 emission source contributing to human health in J city was PM2.5 generated from non-point sources (road dust, construction sites etc.) and the next was NH_3 generated from agriculture (livestock manure, fertilizer etc.). However, contributors of I city showed that NH_3 of agriculture was the prominent contributor and PM2.5 of non-point sources was followed. This study implies that the prominent PM2.5 emission sources should be monitored and well managed to reduce human health impacts due to primary and secondary PM2.5. (This work was funded by the Jeonbuk Green Environment Institute, Korea)

TU213

Improving the life cycle impact assessment of metals in slags: importance of chromium speciation, water chemistry, and metal release

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The investigations of metal ecotoxicity in life cycle assessment (LCA) and life cycle impact assessment (LCIA) are becoming important tools for evaluating the environmental impact of a product or a process. There is however improvement needed for the LCIA of metal ecotoxicity in order to make this assessment more relevant and robust. In this work three issues within LCIA of metal ecotoxicity are investigated, mainly focusing on topics related to stainless steel manufacturing. The first issue is the importance of considering regional water chemistry when constructing the characterization factor (CF) in LCIA. A model freshwater of relevance for stainless steel manufacturing in a region of Sweden was created having water chemistry different from available options. The second issue is related to the lack of consideration of changes in speciation of Cr(VI) in freshwater for a given emission, as Cr(VI) to some extent will be reduced to Cr(III). Two new options were suggested based on general steady-state relationships between Cr(VI)/total Cr as a way to improve the relevancy of LCIA for Cr(VI) in freshwaters. The last issue discusses how to treat metal release from slags in LCIA. Metal release from slags was shown to vary significantly between different ways of modelling slag emissions (total metal content, slag leaching tests, estimated emissions to groundwater). The results show the importance of metal release from slags as it significantly influences the resulting estimated ecotoxicological impact with respect to different CFs that consider Cr(VI) reduction.

TU214

Spatially-explicit characterization of the exposure and health burden of fine particulate matter in the US

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The growing literature on regionalized life cycle impact assessments (LCIA) has highlighted the need to develop spatial estimates to characterize exposure and health damage from pollutants. In this paper, we develop spatially-explicit intake fraction (iF- fraction of the emission taken in by population) and characterization factors (CF – impact per kg precursor emitted) for fine particulate matter (PM2.5) from ground level emissions of $PM_{2.5}$, NH_3, SO_2, and NO_x in the US. We calculate iFs for 43,304 locations in the contiguous US using a reduced-form chemical transport model, the Intervention Model for Air Pollution (InMAP), with high spatial resolution in urban settings and large spatial domain. For each source location, we integrate iFs multiplied by a non-linear exposure-response function and region-specific burden estimates at each receptor location to derive cumulative location-specific CF estimates. The emission-weighted national average CFs using an average slope of the exposure-response are 209 µDALYs/kg for primary $PM_{2.5}$, 94 µDALYs/kg for NH_3, 75 µDALYs/kg for SO_2, and 38 µDALYs/kg for NO_x, with the estimates highly correlated with iF estimates. The US location-specific estimates show considerable variability of about three orders of magnitude. Urban emissions result in higher iF and consequently CF estimates and have a spatial extent about a factor 10 lower than rural emissions. We also report CF estimates for four distinct sectors (agriculture, fuel combustion, industrial processes, and mobile) that account for the spatial distribution of the corresponding emissions, in order to provide exposure and health damage characterizations that are more accurate for processes in life cycle assessment (LCA) studies. Sector-specific estimates for $PM_{2.5}$ and NH_3 vary by about a factor of three with the agricultural sector inducing the lowest and the mobile sector the highest health impacts per kg emitted. This difference is driven by the proximity of emissions to populations. For SO_2 and NO_x, sector-estimates show negligible differences. The sector emission release height also influences CF estimates. This work informs LCIA, helping quantify sector-specific health damages from $PM_{2.5}$–related emissions, using the current state of knowledge for $PM_{2.5}$ exposure and health effect.

TU215

Testing land use biodiversity indicators in organic and conventional beef production systems

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Biodiversity loss has become a major environmental concern closely linked to land use impacts and unsustainable production and consumption patterns. FAO-LEAP TAG on biodiversity indicators aims to provide guidelines and recommendations of which indicators could be suitable to account for the effects of livestock activity on biodiversity. The UNEP-SETAC life cycle initiative has preliminarily recommended the Potential Species Loss, PSL, approach. Following requirements of the UNEP-SETAC life cycle initiative, the method has been improved to include three intensity levels (minimal, light and intense use), as well as reducing characterization factor uncertainty. Both the FAO-LEAP and UNEP-SETAC initiatives ask for case studies to test the performance of indicators with the final aim to show how biodiversity indicators will behave. The goal of the current study is to test applicability and resolution of biodiversity indicators in case studies comparing organic and conventional beef production in Catalonia, Northeast Spain, Europe. We have chosen two extremely different systems, organic and conventional beef production in order to judge information reported by indicators but also feasibility of their application. Total Impact score for the production of 1kg carcass beef are 7.5·10-13 PDF and 2.33·10-12 PDF for conventional and organic production system, respectively. Although, with a very different way of management, the total surface of area used is quite similar per calf, 2834 m2 and 2800 m2 for conventional and organic, respectively. In the organic farm the higher contributors are pastures, mainly because the surface area is larger, but also because of a higher CFs for the ecoregion. In the conventional farm, the most important contributors to biodiversity loss are quantity of soil required to apply manure and area used by the palm feed component. Application of PSLglo biodiversity indicator allowed us to conclude some aspects in relation how the production systems affect biodiversity. However, several aspects still remain not clear enough. Organic farms could have some other biodiversity benefits that are not well represented within the current indicator. The inclusion of other biodiversity taxa more related with soil quality and/or invertebrates could help to improve assessment and might give lower impact values for organic farms. Some of the local biodiversity indicators commented in the coming FAO-LEAP guidelines could contribute to improve the assessment. \n

Bio-Based Industries: Sustainability Benefits of Technological Innovation and Closed Loop Approaches across Supply Chains (P)

TU216

ARE FOREST PRODUCTS CARBON NEUTRAL? BIOGENIC GLOBAL WARMING POTENTIAL (GWPbio) PUT INTO PRACTICE

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Biogenic carbon is widely accepted to be carbon neutral in LCA standards and directives. Carbon lost from forests during wood harvesting is assumed to be recaptured during forest regrowth, and thus the impact of carbon emissions is considered to be zero. Furthermore, the timespan between loss through removal and gain by regrowth is considered negligible with regards to climate change. This assumption has to be reconsidered as the excess of CO_2 residing in the atmosphere does indeed cause radiative forcing effects and hence evidence suggests that this contribution to global warming cannot be excluded. In this work, the radiative forcing resulting from temporary carbon release is quantified. To do this we combined the methodology as used in the calculation of the GWP for a 100-year horizon (CO_2 impulse response function described by the Bern cycle model) with a forest growth curve. This allows to assess the global warming potential from biogenic carbon emissions (GWPbio) of bioenergy produced from existing forests. The GWPbio takes into account several carbon pools such as the soil organic carbon (SOC), the carbon stored in living biomass and natural dead wood. In addition, the natural dynamics of forest stands are considered as well as the potential benefits from delaying the emissions by temporally storing carbon in wood products are considered. Here the carbon footprint results from three case studies are presented: i) bioenergy from forest biomass, ii) kraft paper and iii) construction timber used for housing. These results indicate a significant influence of the temporal increase of the CO_2 concentration in the atmosphere on the carbon footprint results. Nevertheless, the GWPbio factors show a dependency on the sourcing strategy. The values range from almost minus one (thus permanent removal of CO_2 from the atmosphere) for efficient sourcing from fast-growing forests to higher than one for sourcing from slow-growing forests with poor management strategy. This worst-case scenario includes leaving a share of harvest residues left to decay, immediate carbon release through for example burning and the inclusion of foregone carbon sequestration of forests.

TU217

Novel Wood LCA and Environmental Product Declarations

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This work discusses six wood building product CASE studies employing transformational Life Cycle Analysis (LCA) methods. Issues and solutions for modeling spatial variability in land use change, forest form, biodiversity and tropical sources are highlighted. Impact and benefit potentials are compared considering fire-history, fuel type, durability and fate at end of life. Government, Carbon Trusts, NGOs, competing industries and leading economists do have carbon accounting systems that include sequestration. LCA, however, seldom attributes carbon sequestration consistently because of habitat and biodiversity loss. With approaching climate change tipping points, critical time factors demand consistent accounting not only for mitigation but also for carbon drawdown and storage outcomes that include climate braking. Typically the three prior rotations to secure forest product centennial carbon uptake must consider: Previous land use models tropical and sub-tropical deforestation displacing higher value vegetation as well as three prior rotations to compensate for any land use change. Fire and re-growth history to factor carbon loss from soil, detritus and logs. Fuel and scrap use in debarking, saw-milling and wood processing. Fate after wood service life includes reuse and renovation (5-75%), with remaining 90% for recovery and 10% to landfill as incineration is very rare in Australia. Australian and US landfill wood carbon loss and methane generation is much lower than the IPCC base case The range included wood framing, cladding, paneling, paper and board used for 60 years in buildings and 20 years in interior fitout. Results were compared for three building and three fitout products. Ongoing rainforest and fire loss for Brazilian and Malaysian wood cancelled out carbon uptake. Other products had full to minor CO_{2e} uptake, varying with fire loss and biofuel used to debark, sawmill and process logs.

TU218

Biomass waste-to-energy: from residue to resource

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In order to reduce the climate change effects, the final gross consumption of energy in the EU must proceed at least 20% of renewable energies in 2020. Among renewable energies biomass stands out mainly due to its role in the fight against climate change. It has a lower environmental impact and it contributes to improving competitiveness, employment and regional development. In addition, the large number of abandoned forests and the existence of typologies of biomass that are currently not being used make the use of residual biomass as essential to the development of a circular economy. Biomasses that can be valued are those coming from the pruning of vineyards and kiwifruit. They count on a great potential both from the energy and the economic point of view and also high availability. Nowadays, the combustion of biomass in boilers is the most widely used valorization option for thermal applications. However, there are also other forms of exploitation not so widely used, such as cogeneration. This has proven to be an efficient and clean way of simultaneous production of electricity and useful heat in the place of consumption. This technique allows the consequent saving of primary energy (up to 40%) and emissions to the atmosphere. The generation of electrical energy from biomass combustion is based on generating work from heat through steam production on behalf the thermodinamic procces known as Rankine cycle. There is an alternative to the cycle described above, the organic Rankine cycle (ORC). This cycle is similar to the previous one but instead of water it operates with a high molecular weight organic fluid whose boiling point is below 100ºC. The traditional process is considerably simplified in terms of complexity and cost. These advantages make the use of this cycle especially attractive for the use of low quality heat. In this work the viability of the use of microcogeneration (60 kWt boiler-ORC) as a system of energy recovery from vine and kiwi pruning was studied. The results obtained allow to conclude that the temperature differences between the hot and the cold focus influence in a significant way in the efficiency of the employed system. Furthermore, in the conditions used (hot focus temperatures up to 98ºC) cogeneration yields close to 97% (9% of net electrical efficiency and 88% of thermal efficiency) were obtained. Because of this, this method could be postulated as ideal for the energy recovery of the selected residual biomasses.

TU219

Gap analysis of circularity measurements for biological cycles

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The circular economy aims at maintaining material and resources in the economy at their highest utility and value for as long as possible, in order to reduce waste, the material input and the environmental impact associated with it. In many circular economy reports and frameworks, a distinction is made between technical and biological material cycles. The technical cycle involves the management of stocks of non-renewable materials typically extracted from ores, whereas the biological cycle involves the flows of renewable materials, such as wood. However, the existing circularity indicators, focusing on measuring *resource use efficiency* and *recycling rates, are* typically developed for the technical cycle. These indicators seem less relevant to assess the circularity of biological cycles. Technical materials could be restored, refurbished and recycled to their original quality level. In the biological cycle, materials degrade in quality with every subsequent use and hence are cascaded down with every application. Additionally, the biological materials are supposed to return as nutrients to the biosphere, which is not the case with technical materials. Currently, it is assumed that the biomass going back to biosphere contributes to closing the loop. However, this is true only if it maintains the regenerative capacity of the ecosystem. An assessment of a system's capability to foster and sustain ecological cycles is lacking in the context of circularity measurement. There is clearly a gap in the current evaluation of circularity for biological cycles. This study intends to highlight that gap. It focuses on woody biomass, in which case the most often used measure of the cascading use is the *cascading factor*, which quantifies how often the resource is used in a value chain. However, even this measure overlooks factors that are crucial for enabling circularity of biological materials, most importantly the product lifetime and reuse of material. This study aims to assess the characteristics of circularity in wood-value chain, analyze the parameters needed to quantify them and then systematically validate if these parameters are included in the existing circularity measurement tools and indicators. The study also aims to conduct a bibliometric analysis to investigate the different tools and indicators being focused on in the current literature. This gap analysis can then serve as a guideline for designing a thorough framework for circularity assessment.

TU220

Environmental Footprint of bio-refineries feeding with olive biomass residues and wastes

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The bio-economy and circular economy EU strategies promote the use of renewable biological resources with special emphasis to close the loop of wastes. In this new context, sustainability assessment of the bio-refineries using biomass resources, such as those from olive industry, is crucial to select the supply chains that generate the lowest environmental impacts. The aim of this work is to determine the environmental impacts using biomass residues, such as olive tree pruning and wastes residues from olive oil production mill, to produce several co-products in a modern bio-refineries, substituting reference and/or conventional goods, by means of the Environmental Footprint. Two different bio-refineries have been evaluated. One of them uses olive tree pruning as feedstock, while the other one consumes olive pomace residue after extraction of residual oil with hexane in the olive mill. The most relevant results are related to the GHG emissions savings into the global warming impact category, obtaining a range of 51 to 95% when compared to their conventional counterparts. Saving from the land use change effects of removing biomass residues from soil are of the most important contributor to those savings. Nevertheless, due to the application of fertilizers, pesticides and other chemicals in the farming stage, other impact categories such as photochemical ozone formation, acidification, terrestrial and marine eutrophication, present worst environmental behaviour.

TU221

Pros and Cons of the valorisation of fish canning wastewater through biopolymers production

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Fish-canning industries are essential for the local economy in Galicia (NW of Spain) and, at the same time, they generate significant amounts of wastewater with different compositions, which treatment often represents a challenging issue. In this context, the nationally funded projects FISHPOL and TREASURE aim at extracting valuable by-products (i.e. polyhydroxyalkanoates (PHAs)) identified as a promising sustainable alternative to petrochemical plastics (Dietrich et al. 2017)), while treating the wastewater in a sufficient degree to guarantee its safe release to the environment. In parallel, in the recently adopted Plastic Strategy (EC, 2018) the search for alternative feedstocks for plastic production, including bio-based feedstocks, is recommended as long as it is demonstrated that they result in genuine environmental benefits compared to the non-renewable alternatives in a lifecycle perspective. With this perspective the FISHPOL process was developed, which comprises four sequential stages: i) an acidification stage to produce volatile fatty acids (VFAs), ii) an enrichment unit designed to select PHA accumulating microorganisms through a feast-famine regime, iii) an accumulation unit to maximize PHA content inside the bacterial cells and iv) a final extraction process. Therefore, the objective of this study is to evaluate the environmental performance of the FISHPOL process to valorise the fish canning effluents and to compare it with the treatments applied at present: conventional activated sludge and anaerobic digestion completed with an aerobic/anoxic post-treatment. The preliminary Life Cycle Assessment results show a significant impact for the valorisation scenario when compared to the actual scenarios, being the environmental bottleneck the extraction with Dichloroethaneof the biopolymer

from the biomass. An alternative solvent extraction based Dimethyl Carbonate was simulated, resulting in a much lower environmental impact compared to the option applied during lab essays within the FISHPOL project, which positioned the valorisation scenario as a competitive alternative from the environmental point of view. In any case, the evaluation performed has also pointed out the lack of suitable impact categories to consider the effect of plastic littering on water bodies, which is a strong limit of the study, as it leads to the underestimation of the environmental benefit of the biopolymers when compared to their conventional alternative.

TU222

Environmental assessment for fully bio-based self-reinforced polymer composites

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An eco-friendly industry based on green materials, sustainable technologies, and optimal processes with a low environmental impact is today's demand but this remains a considerable challenge to achieve the most appropriate manufacturing methodology. As a fundamental step, there is a need to develop bio-based, sustainable polymeric materials with high stiffness, high impact and high durability without impairing recyclability. The BIO4SELF project within the framework of EU H2020 aims to investigate bio-based Self-Reinforced Polymer Composites (SRPCs). In this work, the environmental assessment of the BIO4SELF project is carried out by applying the Life Cycle Assessment methodology, standardized by the series ISO 14040. The main objective was to analyse the environmental impact of the current production process using bio-based polymers and provide advice on sustainable development of 'hot spots'. As a part of the assessment, the environmental performances of current materials available in the market were compared and the polylactic acid (PLA) that has least energy demand in comparison to other bio-based polymers was chosen. The information provided included the main inputs and outputs, considering energy consumption, materials and generated wastes in the whole life cycle of the production chain of bio-based products. The analysis shows that the highest impact was demonstrated for the PLA granule production, spinning and thermoforming processes due to their higher energy usage. Minor impacts have been observed for commingling, weaving and consolidation processes. The obtained results are in agreement with other bio-polymer based materials (i.e packaging containers). At the end of the project, the achieved results will be updated with the industrial scale production, as the increase in production capacity will lead to the reduction of total energy demand and carbon dioxide emissions. This investigation is expected to open up the path for using PLA materials in a broad range of textile and composite applications. *Acknowledgement* This project has received funding by the H2020 Seventh Framework Programme of the European Union under grant agreement n° 685614.

TU223

Environmental benefits of the green-chemistry based design of Pro-Xylane

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Pro-Xylane is a cosmetic ingredient used mainly as an anti-ageing agent in skin creams. Produced by Chimex, its chemical structure is derived from the xylose, a natural component of glycosaminoglycans, a component of cellulose and an antibacterial and antifungal agent. Pro-Xylane is produced in a two-steps process: 1) The reaction of xylose and acetylacetone in water, then 2) the hydrogenation of the intermediate. The first process developed in 2009 has then been redesign to lower its impacts, following the green chemistry principles, resulting for example in an improved yield, a lower consumption of chemicals, and a change in the catalyst. A life cycle analysis (LCA) has been conducted to compare the first and improved processes. Taking into account all steps of the chemical production, an LCA allows to quantify the environmental impacts, such as air and water pollution, energy use or water consumption. The LCA outcome, based on detailed data collected in Chimex's factory, shows that the eco-design of the process has lead to a large decrease of impacts in main environmental areas of concern, such as the emission of greenhouse gazes. Detailed results will be described during the congress. According to this work, LCA can be used as an useful tool to ecodesign chemical processes, especially when combined with the green chemistry thinking: 1) it helps to understand where the main environmental impacts occur and in consequence, where to focus improvement work, 2) it quantifies the environmental benefits associated with the use of the green chemistry principles.

TU224

Self-healing maintenance road techniques for improving circular economy: Preliminary results of the Environmental Footprint

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Self-healing roads are a new generation of pavements whose materials are capable of recovering their original properties after having suffered breakages or failures. This innovative technology aims to reduce the consumption of natural resources and non-renewable energy in the whole life-cycle of roads, due to the potential achievement of increasing their lifespan compared to traditional maintenance operations. Several previous tests have demonstrated the possibility of achieving new asphalt mixtures by means of the addition of microcapsules that dispense rejuvenating products (that could have bio-oils as components) after heating with microwave or induction energy. Therefore, an ideal scenario would be the self-healing treatment with microwaves or induction when the pavement reaches a certain degree of deterioration, so the traditional maintenance technique could be postponed. Moreover, these new materials could reduce the acquisition of extra abiotic materials for rehabilitations. Thence, it could be a novel technique in line with the redesign and reduction thinking for improving the circular economy. Due to the lack of rigorous environmental and economic studies, the objective of this study is the quantification of the advantages and shortcomings of this novel technique, since it is currently one of the priority research lines for paving materials nowadays. Analyses have been done by the application of the Environmental Footprint (EF) methodology. Preliminary results show that there is a clear influence in the comparative EF for maintenance techniques when rejuvenators are activated by means of microwave-healing technology.

TU225

Life Cycle Assessment of winery by-products valorisation for alcohol and calcium tartrate production: a case study of a real biorefinery in Italy

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The exploitation of winery waste and by-products to produce alcohol and other goods is a step towards wine sector's sustainability in a circular economy framework, although the measure of related environmental impacts is poorly developed. Aim of this study, which is part of the Emilia-Romagna region VALSOVIT project financed by 2014-2020 European Regional Development Fund programme, is to perform a Life Cycle Assessment (LCA) over primary data at commercial scale in a large biorefinery located in Faenza (Italy) to evaluate the impacts produced by treating one tonne of winery by-products (grape marc and wine lees) to produce alcohol and calcium tartrate (CaT), and to compare the results to those of two alternative treatment scenarios (composting and anaerobic digestion). The analysed processes include: 1) grape marc and wine lees handling and distillation; 2) alcohol and CaT production; 3) process residues treatment and valorisation for energy production. The electric and thermal energy used are self-produced by the company via A) the combustion of biogas produced by anaerobic digestion of process residues, and B) the combustion of exhausted grape marc. The excess energy is used in the LCA model to gain credits. Both digestate and aerobic sludge from WWTP are used for agricultural application, gaining credits from avoided production of synthetic fertilizers. The scenarios for comparison have been modelled using secondary data (Ecoinvent and Thinkstep datasets). LCA is conducted using GaBi 8.7 software. The ILCD recommended impact assessment methods and the related characterization factors, along with Primary Energy Demand indicator, have been used for calculating the environmental impacts. The results obtained show that the valorisation of winery by-products is more environmentally performing than both composting and anaerobic digestion, as it is the best option in 8 categories out of the 16 considered in the analysis. For many indicators, such as GWP, Resource Depletion, Water Depletion, Land Use and Primary Energy Demand, environmental benefits are obtained, mostly due to credits from excess energy production. The life cycle phases generating the highest environmental impacts are the grape marc and wine lees processing residues treatment, and the CaT production.

Environmental Risk Assessment of Amphibians and Reptiles: Advancing Knowledge and Reducing Uncertainty (P)

TU226

Effects of pesticides and antibiotics on the early development of the model amphibian organism: Xenopus laevis

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According to the International Union for Conservation of Nature (IUCN), amphibians are suffering a massive decline. With more than 40% of endangered species, they represent one of the most threatened group among vertebrates. Amphibian decline is multicausal and pollution is usually considered as one of major factors involved in population decrease and local extinction. Especially, the use of pesticides and antibiotics in the context of farming activities is suspected to degrade the quality of breeding sites (e.g. lac, swamp, and ponds). Indeed, these molecules are carried by surface and sub-surface runoff as well as drainage into aquatic systems and can be found in high concentrations in surface waters.

Freshwater ecosystems are of crucial importance for amphibians' life cycle. In pond-breeding amphibians, embryonic and larval development takes place in aquatic habitats. Environmental conditions experienced over aquatic development are critical since they affect growth (rate and timing) and survival until metamorphosis. They also have far-reaching consequences for postmetamorphic performances as they influence size and body condition at metamorphosis, two factors directly related to fitness components (survival, growth, and reproduction) at terrestrial stage. Therefore, exposure to chemicals during aquatic development may have a strong detrimental impact on amphibians' population dynamics. To date, the impacts of pesticides and antibiotics on amphibians' populations are still poorly understood. To fill this gap, we investigated the effects on embryos of African clawed frog (*Xenopus laevis*) of nine pesticides and two antibiotics regularly detected in worldwide surface waters. The main objective of this project is to identify and develop sensitive markers for highlighting effect of pesticides and antibiotics at early stages of amphibians' life-cycle. We used the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) as a basis. To improve individual monitoring, we adapted FETAX protocol relying on the Fish Embryo Acute Test (FET), primarily developed for zebrafish. Briefly, embryos are exposed to six concentrations of tested molecules over a 96h post fecundation (hpf) period and four apical observations (coagulation of embryos, lack of somite formation, non-detachment of the tail and lack of heartbeat) are recorded. On top of these observations, molecular biomarkers, embryos' activity, time of hatching and embryos' size at 96 hpf are recorded as well.

TU227

Development of a standardized toxicity test method using a native amphibian

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Although there is growing evidence of the sensitivity of amphibians to contaminants, and thus a growing demand for their use in regulatory frameworks, amphibian toxicity data are currently under-represented in risk assessments. Few standardized methods are available, which in part contributes to this under-representation. In addition, none of the available aquatic methods pair whole-organism chronic endpoints with species that are relevant to Canadian environments. To address this gap, Environment and Climate Change Canada has invested in developing a standardized test method for assessing contaminants with a native amphibian species (*Lithobates pipiens*, Northern leopard frog). We will discuss some lessons learned from laboratory research and data from recent inter-laboratory testing rounds to validate the new test method. For the inter-lab project, we used sodium chloride, thyroxine and perchlorate as model compounds in this multi-laboratory experiment. Environment and Climate Change Canada's amphibian test method will continue along the usual path for standardization of a toxicity test method, including development of quality control criteria, improvement of methodology text, and peer review. The result will be the first Canadian standardized toxicity test method using a native amphibian species.

TU228

Telemetry study on the common toad (Bufo bufo) during postbreeding migration through cereal fields in Germany

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The Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles, recently published from The European Food Safety Authority, points out the need to collect more data on the ecology of these animals to reduce uncertainties when assessing the potential risks associated with exposure to pesticides. Amphibians represent a group of vertebrates endangered at European level but there is still a general lack of knowledge of the ecology of many species, particularly related to their terrestrial life stages. Only a few telemetry studies are available on anurans in Europe and data on their occurrence in different agricultural crops is sparse. Technical developments have now led to reduction of tag weights and an increase in battery life. These developments can now facilitate the undertaking of field studies on amphibian species aimed at providing data to determine relevant PT values (i.e. the portion of time individuals spend active in treated crops) for risk assessment purposes. We will present data for the common toad (*Bufo bufo*), obtained from individuals equipped with state-of-the-art radio tags in order to follow the animals in the post breeding season. The methods used and results obtained will be presented and discussed.

TU229

Telemetry of sand lizards (Lacerta agilis) in vineyards - do methods established for terrestrial vertebrate risk assessment to record higher tier data work for reptiles?

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The scientific opinion on the state of the science on pesticide risk assessment for amphibians and reptiles, published in 2017 by the European Food Safety Authority, highlights a gap in our knowledge of the ecology of the reptiles to be used for higher tier risk assessment. The sand lizard (*Lacerta agilis*) was proposed as a focal species to represent other lacertids utilizing agricultural land. *L. agilis* is commonly distributed in vineyards, a permanent crop to which pesticide applications are frequently made. For sand lizards, vineyards represent what can be considered to be a secondary habitat. *L. agilis* occurs predominantly at the edges of these areas, particularly if bordered by abandoned gardens. A study site with these characteristics was selected, as these habitats represent areas from which the animals can move into the surrounding vineyards. Several sand lizards were caught and individually marked. Continuous radio-tracking was performed in summer 2018 and confirmed the use of vineyards and other habitats by *L. agilis*. The applicability of refinement factors recommended in the scientific opinion on the state of the science on pesticide risk assessment for amphibians and reptiles could be therefore demonstrated. These refinement factors included the portion of time active animals spend in treated areas, as commonly applied to other terrestrial vertebrates. In addition to data on the use of vineyards by sand lizards we also present the tagging methodology used and other technical aspects of the telemetric process.

TU230

Examining Mercury Transfer in the American Alligator (Alligator mississippiensis)

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Prenatal exposure to environmental contaminants is a cause of developmental issues in humans and wildlife. Mercury (Hg) and other environmental contaminants are particularly detrimental to the developmental phases, often impairing the organization and development of important organ systems by causing irreparable damage. Wildlife species that are environmentally exposed to elevated Hg concentrations can be useful in modeling human exposure. The transfer of Hg in the American alligator (*Alligator mississippiensis*), a common sentinel for contaminant exposure, was examined in a series of experiments. To determine the presence of Hg within the eggs, total Hg (THg) was measured by atomic absorption spectroscopy in alligator egg yolk samples from three locations, Yawkey Wildlife Center, SC, Lake Apopka, FL, and Lake Woodruff, FL. The mean THg concentrations observed at each site were 26.3 ng/g ± 11.0 ng/g, 8.8 ng/g ± 5.1 ng/g, 22.6 ng/g ± 6.3 ng/g, respectively. After the presence of Hg in alligator egg yolk was confirmed at different locations, maternal transfer was examined by measuring THg in nesting female blood samples, corresponding egg yolk, and embryo samples across three years from a single location, Yawkey Wildlife Center, SC. We observed that ~12 % of the maternal THg is found in their egg yolk, and a highly significant correlation was observed which explained 66% of the overall variation between the two samples ($rho = 0.66$; $p < 0.0001$). The correlation equation derived by this analysis ($y = 0.138x – 2.5534$) was the used to extrapolate maternal transfer in alligator populations at other locations based on the nesting female's blood THg, and further evaluate alligators a developmental model for embryonic Hg exposure. To assess environmental Hg transfer the developmental compartmentalization of topically applied THg was examined using alligator eggs collected from a low Hg site, Lake Woodruff, FL. Through this analysis, we observed that environmental Hg transfer is unlikely after calcification. The observed nest-specific THg concentrations that persisted through the dosing experiment further indicate that maternal transfer is occurring. This study details new life history information for the American alligator, which can improve biomonitoring efforts and inform population management programs in areas of high Hg contamination, as well as identifying this species as a candidate model organism for maternal transfer of environmental Hg exposure.

TU231

SETAC Ecotoxicology of Amphibians and Reptiles Interest Group

C. Aubee, U.S. Environmental Protection Agency / Office of Pesticide Programs

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Wildlife Ecotoxicology: From Sub-lethal Responses to Adverse Effects at Individual and Population Level (P)

TU232

In vitro modulation of transcriptional activity of nuclear receptors of fin and blue whales by environmental pollutants

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Baleen whales, such as fin whales (*Balaenoptera physalus)* and blue whales (*B. musculus*), are impacted by multiple stressors, including exposure to pollutant mixtures. Both of these species are listed in threatened categories in the IUCN Red List of Threatened Species. Despite their conservation status, there is little information available regarding persistent organic pollutant (POP) concentrations in their blubber, or the potential toxicological effects of pollutants on these species. One critical mode of action of POPs is endocrine disruption, but this aspect of toxicology has been studied very little in wildlife. The health of marine mammals depends on the normal functioning of energy metabolism, the immune system and reproduction. These processes are regulated by nuclear receptors, such as thyroid hormone receptor beta (THRB), glucocorticoid receptor (GR) and the peroxisome proliferator-activated receptor gamma (PPARG). To study the transcriptional activity of THRB, GR and PPARG when exposed to legacy POPs that are abundant in whale blubber, GAL4-UAS based *in vitro* luciferase reporter gene assays were performed. PCB52, PCB118 and *o,p´*-DDT increased the transcriptional activity of GR. The transcriptional activity of PPARG was increased by PCB congeners 101, 118, 138, 153 and *o,p´*-DDD, *trans*-nonachlor, oxychlordane and the POP mixture. THRB transcriptional activity was increased by PCB153 and antagonistic effects were detected for *trans*-nonachlor, PCB52, oxychlordane, PCB101, PCB138, *o,p´*-DDE and HCB. These results are also representative for other mammalian species, because the ligand binding domain (LBD) of THRB has been shown to be identical in killer whales, white whales and humans; PPARG-LBD is the same in killer whales, white whales, polar bears and humans and the LBD of GR is the same in killer whales and minke whales. The findings of the study contribute to a better understanding of an endocrine disruptive mode of action of POPs on whales and other mammals. Due to remaining knowledge gaps, additional studies will be needed that focus on the effects of POPs on endocrine disruption.

TU233

Field study in apple orchards to assess exposure and effects of a fungicide on the European Rabbit (Oryctolagus cuniculus)

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The EU requires a risk assessment for non-target wild mammals to register a Plant Protection Product for the European market. Here we present a realistic field study on populations of the European rabbit (*Oryctolagus cuniculus*) to monitor for potential effects after sprays of a fungicide in apple orchards. The aims were to elucidate: (i) the presence of rabbits in the apple orchards, (ii) their exposure to the fungicide by grazing on the grassy understorey and (iii) any effects on reproduction by comparing test-item-treated orchards with reference orchards. The study was conducted in six apple orchards in Herefordshire, UK. It ran from March to August (2018) which is the period of rabbit reproduction in the UK. The cropped area of three orchards was treated with 10 applications of a fungicide. The other three orchards were not treated with the test-item and hence served as reference sites. The analysis of the vegetation samples found residues of the test-item in the grasslayer between the tree rows after the applications. Thus, grazing rabbits were exposed to the test item. To document the presence and behaviour of rabbits thermal-imaging devices were used during ‘Transect Counts’ inside the orchards. In each orchard 13 transect counts of approx. three hours length each were conducted. Rabbits could be observed during all transect count sessions and in all six orchards the abundance and behaviour of observed rabbits showed no significant difference. Spatial data from 15 adult females tagged with a GPS-collar continuously recorded location data between two to eight days per individual. The data revealed that their warrens were located in the margin of the orchards. Moreover, the data showed that all tagged rabbits spent a considerable amount of time within the test item orchards. From these results it could be concluded that the rabbits inhabiting these warrens used the orchards for foraging and were, therefore, exposed to the test item. In order to identify the proportion of juvenile rabbits in the local population, as an indicator for reproduction, ten automatic ‘Wildlife Cameras’ were placed in each orchard. 39,576 video footages showing at least one rabbit were analysed for the number of juveniles and adults visible. There were no statistically significant differences in juvenile proportions between ‘test item orchards’ and ‘reference orchards’. Hence, there was no indication that rabbit reproduction was affected by the test item application.

TU234

Validation of dynamic energy budget physiological models for small mammals

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In the prospective environmental risk assessment for pesticides, toxicokinetic-toxicodynamic (TK-TD) models are useful tools for the evaluation of potential risks and may help to reduce animal testing. Dynamic energy budget (DEB) models are particularly suitable to analyses chemical effects on life-history traits related to growth and reproduction. DEB models for chemical effects consist of two modules, a ‘physiological’ module that describes the energy acquisition and use for growth and reproduction, and a TK-TD module that accounts for the chemical effect. While the TK-TD module is always specific for a given chemical, the general idea of the physiological module is that the same DEB model can applied to the different species, and species only differ in their parameter values. Model validation is key to reliable applications within the prospective environmental risk assessment. We will showcase a validation of the physiological DEB model for two laboratory species, the Wistar strain of the brown rat and the New Zealand White strain of the European rabbit. Both model parametrizations are publically available from the add-my-pet collection which generally hosts underlying code, parameter estimates and data for a broad range of species.

TU235

Determining the effects of 3 nonsteroidal anti-inflammatory drugs (NSAIDS) on the african clawed frog Xenopus laevis

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NSAIDs are drugs most consumed worldwide because used to remedy the pain of mild to medium grade and they are over-the-counter. These drugs are present in hospitals effluents. and has been demonstrated that these drugs do not degrade in water treatment systems, for this reason have been detected in surface water at concentrations of 6.5 ng L^{-1} to 100 ug L^{-1}. Because the effects of these drugs on aquatic organisms are not known in this work, the oxidative, neurotoxic and genotoxic effects of 3 analgesics: Naproxen, Paracetamol and Acetylsalicylic Acid was evaluated in the African frog *Xenopus laevis*. X. laevis specimens were obtained by donation. They were grown in the laboratory under the following conditions: Temperature 23 ° C, Photoperiod: 16 hours Light/8 hours Darkness. Water hardness: 160 mg. With juveniles of 3 cm in length, bioassays were performed where 6 organisms were exposed to a sublethal concentration of the drugs (CL_1) for 15 days. Skin samples were taken for the determination of lipoperoxidation (Tbars), brain to determine the activity of the enzyme acetylcholinesterase (AchE) and blood for the evaluation of micronuclei frequency. A "t" test was carried out between the control and the exposed organisms to detect the differences in the responses to the drugs. In the results obtained, significant differences were observed between the control and the organisms exposed to NSAIDs. The analgesic with the highest oxidant effect (12.6 nM Tbars mg^{-1}) was paracetamol (control = 0.94 nM Tbars mg^{-1}). A decrease in AchE activity of more than 20% was observed in the Naproxen tests. The 3 drugs had genotoxic effects. The highest frequency of micronuclei was obtained in the tests with paracetamol (4.2%) and the lowest frequency in the tests with acetylsalicylic acid (2.5%). The results indicated that in sublethal concentrations tested NSAIDs have deleterious effects on frogs. It is important to study the effect of these compounds in aquatic systems, to determine the degree of risk in which organisms are chronically exposed to these drugs.

TU236

Virus infections are augmented by exposure to environmental pollutants in vertebrates

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During the last few decades we bear witness to the emergence/re-emergence of virulent pathogens that threaten human and wildlife health. Despite advances in biomedical research and improvements in therapeutics, virus infections still cause significant morbidity, mortality and socioeconomic loss. Among the most cited and recognised drivers of pathogen emergence are climate change and habitat destruction, with other clear factors including viral subtype, host genetics and age. Yet, these factors do not completely explain the variation among responses within an infected population. Other exogenous factors thereby must play a role. Here we review how legacy pollutants, that are known to modulate immune function of vertebrate hosts, affect the emergence/re-emergence of virus infections and may thereby drive outbreaks and the evolution of disease severity. The immunomodulating properties of such chemicals have led to postulations as early as the 1960s that they are involved in viral outbreaks in humans and marine mammals, as well as increasing the severity of virus infections in a number of animal models. Despite these presumptions, a clear link between pollutants and virus infection severity has yet to be determined. Thus, we reviewed the literature on the effects of legacy pollutants on virus infections in vertebrate hosts. Here we present the results of this review which provide the first review and meta analysis on the effects of legacy pollutants on virus infections in vertebrates. We show that pollutants augment mortality and virus titre in infected mammals, fish, and birds.

TU237

Immunotoxicology of persistent organic pollutants and mercury in northern Norwegian White-tailed eagle (Haliaeetus albicilla) nestlings

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The marine environment functions as a sink and transport route for contaminants, and marine top predators are characterised by relatively high levels of organohalogenated contaminants (OHCs) and mercury. As such, they represent good biological models to assess the impact of such environmentally persistent pollutants (EPPs) on ecosystem health. Here we propose a project that will use a sentinel top predator in the Norwegian marine fauna: the white-tailed eagle (WTE: *Haliaeetus albicilla*) for developing new methods to understand how EPPs affect biomarkers of immunotoxicity, i.e., leukocyte coping capacity (LCC). LCC assesses the capacity of circulating leukocytes to produce a respiratory burst *in vitro* in response to a secondary challenge by phorbol myristate acetate (PMA). This measurement was performed in the field on WTE nestlings in three different areas of northern Norway and represents an *in vitro* assessment of the bird's current physiological status and its ability to cope with stressors. Individuals with a higher LCC have a greater potential to produce a respiratory burst, and physiologically, are better able to respond to challenges after stress. In the context of immunotoxicity, we predict both inter-individual and inter-population differences. First, we predict that WTE nestlings with a higher load of contaminants will have a reduced LCC (i.e., lower immune defense and higher susceptibility to stressors) compared to less contaminated nestlings. Second, we predict that there will be regional differences in LCC-profiles, considering that environmental quality (e.g. habitat quality and food resource) influence the level of contaminants and stressors in the chicks. Preliminary results show evident differences in LCC profiles among WTE nestlings, and these differences will be further investigated in relation to level of OHCs in blood plasma and mercury in feathers, in addition to analysis of stable isotopes in feathers to include effect of diet. Final results from this study will be ready by early May 2019.

TU238

More than one can bear: Exposure to persistent organic pollutants alters the adipose tissue transcriptome in polar bear mother-cub pairs from Svalbard, Norway.

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Being on top of the food chain, polar bears (*Ursus maritimus*) are highly contaminated with persistent organic pollutants (POPs). Females nurse their offspring with contaminated milk – consequently, even young cubs have higher POP concentrations than their mothers. Recent studies suggest that POPs may affect lipid metabolism in female polar bears, however, the mechanisms and impact on her offspring remain unknown. We hypothesize that (1) increasing exposure to POPs in polar bear mothers disrupts adipose tissue transcriptomes of adult female polar bears and their offspring and (2) POP-related transcriptomic dysregulation in adipose tissue is sex-specific and differs between male and female polar bear cub pairs.Adipose tissue biopsies were collected from adult female polar bears and their cubs in Svalbard, Norway, spring 2011-2013 (PCB plasma concentration: 10.46 - 248.62 ng/g wet weight). Total RNA extracted from biopsies from adult females (n=13) and cub pairs (?-?n=5 | ?-?n=4 | ?-?n=4) were subjected to next-generation sequencing to assess RNA presence and quantity.The expression of the top 50 most correlated genes with POPs exposure are altered differently due to low, medium and high exposure in mothers. Several cubs from low exposed mothers had a gene expression profile resembling medium exposed cubs. Male and female cub pairs responded differently to POPs. DISCUSSION & PERSPECTIVES: POPs alter the adipose transcriptome of polar bear mothers and cubs, which may be linked to metabolic dysfunction. Male and female cubs demonstrated remarkably different adipose transcriptome profiles.We will elucidate coping mechanisms polar bears utilize to adapt to their changing environment. This is also a unique opportunity to observe sex-specific coping between cub pairs. This study will broaden our understanding of POPs and their impact on lipid metabolism and physiology in apex predators and offspring.

TU239

Large-scale monitoring of the exposure to per- and polyfluoroalkyl substances and mercury in the white-tailed eagle (Haliaeetus albicilla): clinical-chemical effects and the role of diet

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Although bird of prey nestlings are considered valuable sentinels of local environmental contamination, an effort to evaluate their extended suitability for large-scale monitoring was yet to be endeavoured. We have therefore sampled blood and body feathers from white-tailed eagle (*Haliaeetus albicilla*) nestlings along the Swedish, Finnish, Estonian, German, Danish, Norwegian and Icelandic coasts. This large-scale effort represents the anticipated exposure spectrum and has documented individual traits (age, crop content, foraging habitat and trophic level) that may confound contaminant exposure and effects. Body feathers and blood plasma were analysed for mercury (Hg) and per- and polyfluoroalkyl substances (PFASs), respectively, and a range of blood clinical-chemical parameters (BCCPs) was quantified as well. Finally, stable carbon (foraging habitat) and nitrogen (trophic level) isotopes were analysed in the same body feathers. Our first results confirm elevated exposure to Hg and PFASs in Baltic nestlings and lower exposure in Norway and Iceland. Notable Hg hotspots are located along the Swedish coast and in Finnish Lapland, while only the central Swedish and Finnish Baltic seem to be hotspots for PFASs. We did not observe a significant overall relationship between trophic level and contaminant exposure, which supports the notion of locally elevated environmental background levels. Multiple regression models investigating the simultaneous effects of individual contaminant exposure, age, short-term food ingestion, foraging habitat and trophic level indicate that the latter two stable isotope based dietary descriptors also show relationships with BCCPs indicative for liver, kidney and blood metabolism. Moreover, short-term diet ingestion (recorded crop content) as well as an individual's age influences these same BCCPs as well as a range of elements. Finally, the models show the potential effect of Hg and PFAS exposure on clinical health as significant relationships where found between the contaminant levels and BCCPs indicative of liver and kidney functioning. We will present a more thorough data analysis of this expanding dataset in order to show the feasibility and importance of large-scale monitoring, which allows evaluating the efficiency of dedicated contaminant Conventions as well as the impact of increasing environmental change.

TU240

Blood clotting assays enhance assessment of anticoagulant rodenticide exposure and effects in free-ranging birds of prey

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Non-target wildlife, particularly birds of prey, are widely exposed to and acutely poisoned by anticoagulant rodenticides (ARs). An unresolved issue surrounding such exposure, however, is the potential for sublethal effects. In particular, the consequences of AR exposure and resulting coagulopathy on health and survival of unintentionally exposed animals, which often encounter a multitude of anthropogenic stressors, are understudied. In a wildlife rehabilitation setting, AR intoxication may be masked by more obvious injuries related to collision with vehicles or electrocution, thereby obfuscating proximate from ultimate cause of mortality , although the two make also act cumulatively to cause death. An assessment of coagulation function of admitted wildlife may provide a means of establishing the proportion of animals exhibiting sublethal coagulopathy, and ultimately ensuring provision of appropriate and swift treatment. In conjunction with routine diagnostics for injury and disease, we performed two blood clotting assays (prothrombin time, Russell's viper venom time) affected by vitamin K-dependent coagulopathy of samples from live raptors admitted to a rehabilitation facility. We also measured clotting time in pre-fledge barn owl chicks from 10 nest sites in Lower Mainland Canada. Prolonged clotting time or failure to form a clot altogether was observed in 23% of 61 sampled raptors admitted to the rehabilitation facility. This is a biologically significant proportion of individuals given the fortuitous and biased nature by which raptors are found and admitted to rehabilitation facilities. In contrast, there was no evidence of coagulopathy in any of the pre-fledgling barn owl chicks. The utility of avian coagulation tests for diagnosing AR exposure is promising, yet there remains a need to establish species specific reference values and standardize and streamline[assay methodologies among test facilities.

TU241

In vitro cell proliferative effect induced by organochlorine pesticides and their binary mixtures detected in raptors from Southeastern Spain

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The effects related to the exposure to organochlorine pesticides (OC) have been widely studied in many species, including human and wildlife, around the world. Over the past three decades, studies on their effects have been focused on endocrine alterations in living beings, for example, estrogenic effects. Wild birds, especially raptors, have been widely used as sentinel of both environmental exposure to OC, and their acute and chronic effects. However, there is still a great uncertainly about the exposure to OC mixtures. In this study we have used a rapid and sensitive in vitro test to evaluate cell proliferation in MCF7 cell line after exposure to increasing concentrations of four OC pesticides (p,p'-DDT, p,p'-DDE, endosulfan αβ, HCH-technique) and four binary mixtures (two of them at 1:1, DDT:DDE and endosulfan:HCH; and the other ones at 3:1, endosulfan:DDT and endosulfan:DDE). Mixtures were designed following patterns of exposure to OC in raptors species from the southeastern Spain. MCF7 cells were exposed to 7 sublethal concentrations of each pesticide and each mixture, using the 6-day E-Screen assay. 17β-estradiol was used as positive control, and DMSO as vehicle. The range of assessed concentrations was 1,0E-5M to 1,0E-11M for individual compounds, and diluted until 1,0E-13M for mixtures. Relative Proliferative Effect (RPE) referred to the positive control allowed us classify each compound and mixture as weakly (RPE< 25%), partially (25-75%) or highly (>75%) estrogenic. All single OC were classified as highly estrogenic, at least, in one of the concentrations assessed, although the intensity on their responses varied along the concentrations. RPE obtained for the mixtures were lower than those for single compounds, except for the endosulfan:HCH mixture, which was classified as highly estrogenic. Moreover, the highest effect was observed at the lower concentration. Half of the binary mixtures were weakly estrogenic, and the rest showed partial or high cell proliferative effect. Finally, we suggested that difference on affinity of each compound with each estrogenic receptor seems to be more relevant than its relative presence in the mixture. Keywords: Estrogenic, proliferation, organochlorine, mixture, in vitro, MCF7, raptor.

TU242
Eggshell index, declining fertility and embryonic death could be related to the presence of DDE and dieldrin in unhatched eggs of Montagu's harrier (Circus pygargus)
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Unhatched eggs have been widely used to assess exposure and effects induced by environmental endocrine disruptors, especially organochlorine compounds in raptors. Data on exposure estimates of the mother, body burden of contaminants in nestlings and reproductive success, among others, could be easily obtained from this type of sample. However, eggs also have some disadvantages, like the scarce number of available samples to be analysed. On the other hand, the effects of DDE on the reproductive success in birds of prey have been well reported. From the beginning of this century, Montagu's harrier (Circus pygargus) population dramatically declined in Southern Spain. To try to relate it to exposure to organochlorine pesticides, 15 compounds were analysed in the contents of 55 Montagu's harrier unhatched eggs sampled in Southern Spain between 2003-2006. The pesticide concentrations were studied in relation to different parameters of fertility, embryonic death and reproductive success. Threshold effect levels related to reproductive success were also estimated for DDE and dieldrin. Eggshell thickness (ET; mm) was measured and Eggshell Index (EI; g/ mm2) calculated in all eggs. P,p'-DDE showed the highest mean concentration (14.4 mg/g lipid; median 8.56 mg/g lipid) and was also the most frequently detected (in all fertilized eggs, and in 97% of unfertilized eggs). Lindane, heptachlor and its epoxide, endosulfan I, and dieldrin were also detected but at lower concentrations. Fertilized eggs showed higher concentrations of pesticides, especially of p,p'-DDE and lindane, than unfertilized eggs, with the exception of dieldrin which showed a mean concentration ten times higher in unfertilized than in fertilized eggs. No relationships between pesticide concentrations and ET nor EI were found, except for p,p'-DDE and EI (r=-0.530; p=0.006). Eggshell index could be a better biomarker of hatching failure than ET. Finally, two eggs showed p,p'-DDE concentrations above the threshold level related to reproductive effects proposed by other authors. After comparison with other studies, we propose 3000 ng/g (wet weight) of p,p'-DDE as NOAEC in Montagu's harrier. Regarding dieldrin, the highest concentration was 136 ng/g (wet weight), 3-6 times lower than the threshold values proposed by others, but a relationship between dieldrin exposure and fertility should not be discarded. **Acknowledgments**: EGMASA-Junta de Andalucía, ERBFacility-Cost Action.

TU243
Monitoring long term effects of a crop protection product on birds: a case study in citrus orchards in Italy
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According to the Guidance Document on Risk Assessment of Birds and Mammals (EFSA 2009) potential adverse effects of pesticides on wild vertebrates should be assessed. One higher tier refinement option for wildlife is to conduct field effect studies to monitor potential acute and long-term effects on birds. In the past, it was not questioned to move application timings (intended according to the GAP in summer or autumn) into spring: It was commonly agreed between applicant, regulator and other parties involved in study planning, that applications (i.e. exposure of birds) were set to the onset of breeding, and that this would be a worst-case scenario to explore potential long-term (i.e. reproductive) effects of exposed individuals. However, within the last few years a regulatory concern of this design becomes more common that short-term exposure at any time of the year could have long-term effects on reproduction. The current study aimed to investigate potential long-term reproductive effects on exposed birds after insecticide use against Mediterranean fruit flies (*Ceratitis capitata*) and to cope with the logistic difficulties to follow exposed birds between breeding seasons. The study was conducted based on member state requested design in 2016 and 2017 in Sicily, the most representative region for citrus in Italy. The product is usually sprayed when the fruits grow, thus in late summer when the breeding season of birds ends. We focussed on the most representative species such as sparrows (*Passer sp.*), blackbird (*Turdus merula*), goldfinch (*Carduelis carduelis*), great tit (*Parus major*), Sardinian warbler (*Sylvia melanocephala*), utilizing such citrus orchard, and covering all diet guilds of birds using orchards. We applied bird trapping, nest searching, monitoring and telemetry as field methods. There was no evidence for any long-term effects such as reduced breeding success or mortality of young reared by exposed individuals of the previous season in treated citrus orchards. This study and its results can be considered an example how registration relevant reproductive data can be recorded even for birds exposed late in the season, when the breeding period is already completed.

TU244
Effect of exposure duration on the magnitude of body weight effects in mammalian toxicology studies
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The time-dependence of effects on female body weight observed in dietary toxicity studies conducted with rats was evaluated using Benchmark Dose calculations (BMD_{10}, i.e. an effect level of 10% was considered). A total of 37 long-term toxicity studies conducted with 13 different active substances used as pesticides were evaluated. These active substances belong to different use classes (i.e. herbicides, insecticides and fungicides). Time-dependent changes of BMD_{10} were evaluated for each active substance using female body weights after 14, 21, 28, 42 and 70 days of exposure. The greatest body weight effects were observed at the end of toxicity studies (after longest exposure). BMD_{10} values declined continuously with exposure duration for all use classes and substances. After 70 days of exposure the BMD_{10} levels were about half of the BMD_{10} after 14 exposure days, indicating that animals respond to pesticide exposure in an exposure-time-dependent way, i.e. effects on body weight of the animals are less pronounced when the duration of exposure is short. Based on these findings, the realism of the current wild mammal risk assessment for plant protection products is discussed and how it could be improved by considering an appropriate time period for the selection of endpoints in chronic toxicity studies, which reflects the exposure time of free ranging animals in the field.

TU245
Essential and non-essential chemical elements in the muscles and liver of harbour porpoises (Phocoena phocoena) from the Norwegian coastal waters
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Anthropogenic activity and the associated industrialization have been often accompanied with pollution of marine environment. Heavy metals and other potentially toxic elements may contribute to adverse health effects of marine mammals. Moreover, concentration of elements in organs and tissues of top marine predators may integrate information about general pollution status of marine waters. The aim of this study was to examine the influence of biological and environmental (seasonal variation) factors and spatial distribution in relation to potential source on the accumulation of elements in liver and muscles of harbour porpoise. Therefore, in 2016 and 2017, 134 by-caught specimens of harbour porpoises (*Phocoena phocoena*) were collected along Norwegian coast. Concentrations of 47 chemical elements were quantified by ICP-MS. In comparison to other areas, relatively low concentrations of hazardous elements were found in the muscles and livers of Norwegian porpoises. Increasing levels of Cd and As determined in populations from northern part of Norway could be related to their distinct feeding preferences. For Hg concentrations, there were significant relationships with Ag and Li in hepatic and muscle tissue, whereas Se was strongly correlated ($p < 0.001$) with most of toxic elements such as Ag, Cd, Hg, Pb, Sn. For Cu, statistically significant negative relationships with Ag and Cd were probably related to competitive binding to metallothionein. The body length was found to be significantly ($p < 0.001$) related to Ag, Au, Bi, Cd, Ce, Co, Cs, Hg, Mo, Nd, Pb, Pr, Sb, Se, Sm, Sn, V, Zr, whereas Ca and Cu revealed negative significant relations to body length. Au, Ag, As, Cu Ba, Cs in liver and Ti, Cu, Sb, Rb in muscle was higher in females than males, whereas males had higher content

of Se and Ni in liver and Sn in muscle. Hepatic tissue generally had higher elemental concentration than muscle, with exceptions for Al, Cr, Cs, K, Mg and Ni. The results on spatial differences indicate northward increasing abundance of Cd and As in harbour porpoises along the coast of Norway. For several elements there were also seasonal differences between spring and autumn, however the reason for this is yet to be explained.

TU246

DNA double strand breaks and chemical elements in incubating female common eiders (Somateria mollissima) in Christiansø, Denmark

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Genotoxic agents are ubiquitous in the Baltic Sea and may cause effects not only at a molecular level but at an individual and population level as well. Fasting during reproduction may lead to a state of oxidative stress and enhance the genotoxicity of non-essential elements due to low levels of essential elements and nutrients. Therefore genotoxic agents pose a threat to fasting species. The present study aimed to assess DNA double strand breaks (DNA DSBs) in relation to body mass and non-essential elements in blood of female common eiders (Somateria mollissima) in the Southern Baltic Sea (Christiansø, Denmark) at the beginning (day 5) and end (day 25) of incubation. Furthermore, the present study aimed to investigate the relationships between non-essential and essential elements in whole blood of female common eiders (*Somateria mollissima*) on day 5 and day 25 of incubation. This was a unique study because each incubating eider was sampled twice and therefore acted as its own control. The present study analyzed DNA DSBs using gel electrophoresis to quantify DNA-fraction, of total DNA, that migrated into the gel (DNA-FTM). During incubation DNA-FTM increased significantly throughout incubation (0.4 -70 %). Body mass decreased significantly (17 - 44 %) throughout incubation. Significantly increasing levels of Cd were associated with decreasing levels of Ca and Zn and increasing levels of Cu, which may demonstrate an increase in absorption of Cd from day 5 to day 25 of incubation. Increasing levels of Pb were significantly correlated with decreasing levels of Ca, which may indicate Pb was released from medullary bone during incubation. As and Hg were not found to significantly increase. Hg was found to be positively and significantly correlated with Se, suggesting a protective effect of Se on Hg. DNA-FTM was found to be negatively and significantly correlated to body mass and positively correlated to Hg (not significantly). Given the high levels of DNA DSBs in the current study compared to previous studies in Baltic Sea eiders, there may be other factors at play, apart from non-essential elements, causing DNA DSBs. However, the high levels of DNA DSBs and body mass loss may reflect the overall health of this endangered population, which is exposed to multiple stressors.

TU247

Toxic metals and rare earth elements in blood of red-necked nightjars (Caprimulgus ruficollis) inhabiting differently polluted environments

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Toxic metals have been widely reported in avian tissues due to their well-known accumulation capacity and the adverse effects they cause in vertebrates. However, further research evaluating metal exposure is required to assess spatial and temporal trends and fill the knowledge gap for some species such as the red-necked nightjar (*Caprimulgus ruficollis*), from which, to the best of our knowledge, no data exist. In addition, rare earth elements (REE) and other minor elements (ME) are becoming increasingly important due to their use in modern technology, but data on exposure and effects have been rarely reported, with no studies in wildlife. This study reports concentrations of 50 elements (i.e. trace elements, ATSDR's list toxic elements, REE and ME measured by ICP-MS) in whole blood of red-necked nightjars inhabiting three different scenarios of contaminant exposure (agricultural/urban area, n=15; ancient mine site, n=17; and control area, n=16; in south-eastern Spain). Levels of the essential elements Se and Mo were higher in nightjars inhabiting the control and the agricultural-urban areas compared to the ancient mine site. However, birds inhabiting the mining area showed higher concentrations of the toxic elements As, Pb and Cd, and lower hematocrit level. Decreased Se levels in the mining area may be related to its protective role against metal toxicity. Several essential elements (Fe, Zn, Cu, Se, Mn, Mo, Cr, Co, Ni) were positively correlated with different toxic metals (e.g. As, Cd, Hg, Pb, Ba, Sr, V), REE (e.g. Ce, Ga, La, Nd, Y) and ME (e.g. Ti). Hematocrit was negatively correlated with Pb, Cd and Sr; moreover, Pb and Cd concentrations were similar to or higher than levels reported as causing adverse effects on the antioxidant system in other avian species, suggesting that nightjars in the ancient mine site may be suffering from biochemical effects related to toxic metal exposure. Even though most REE and ME concentrations were close to the LOQ, some of these emerging contaminants may trigger sub-lethal effects that, together with the ATSDR's list toxic elements, will be evaluated in a future study.

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TU248

Blood concentrations of toxic metals and rare earth elements from E-waste in nestling Eagle owls

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Toxic metals accumulate in the organism and their adverse effects in vertebrates are well documented. However, rare earth elements (REE) and other minor elements (ME) increasingly used in technological and electronic devices have been rarely reported, with no studies in wildlife. This study reports for the first time blood concentrations of 50 elements, including essential trace elements (Fe, Zn, Cu, Se, Mn, Mo, Cr, Co, Ni), ATSDR's list toxic elements (Ag, Al, As, Ba, Be, Cd, Hg, Pb, Pd, Sb, Sn, Sr, Th, Tl, U, V), REE (Ce, Dy, Er, Eu, Ga, Gd, Ho, In, La, Lu, Nb, Nd, Pr, Sm, Ta, Tb, Tm, Y, Yb) and ME (Au, Bi, Os, Pt, Ru, Ti) measured by ICP-MS in nestling Eagle owls (*Bubo bubo*) inhabiting three different scenarios of contaminant exposure (agricultural/rural area, n=50; industrial area, n=14 and ancient mine site, n=23; SE Spain). Some essential element concentrations (Zn, Se, Mn, Co, Ni) were higher in nestlings inhabiting the agricultural/rural area. Nestlings showed higher concentrations for some ATSDR's list toxic metal (As, Hg, Pb, Th, Tl), REE and ME (Ta, Os) in the ancient mine site. Hg and Pb are known to alter the metabolism and function of essential elements, which could explain the negative correlations found (Hg-Fe, Hg-Mn, Hg-hematocrit and Pb-Co) in the mining area. A previous study showed that Pb, Cd and Hg at similar exposure level disrupted antioxidant molecules in this species. This study shows that, in addition to the classical toxic elements, Eagle owls are exposed to other ATSDR's list elements, ME and REE, the latter included as new and emerging contaminants by international organizations. Toxic effects of long-term exposure to environmentally relevant levels of such substances are poorly documented and should be evaluated in future studies.

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TU249

Bird feces as indicators of metal pollution

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Non-invasive sampling methods are urgently needed for ecotoxicological field studies. Birds have turned out to be good biomonitors and bird feces have been used as a proxy for measuring dietary metal exposure levels in wild populations. Collecting and analyzing feces from nestlings is an easy and cost-effective way of getting information on individual dietary exposure. However, what can be concluded on fecal levels is not always clear and there are several potential methodological pitfalls. We have started a series of studies aiming to improve the reliability and repeatability of fecal metal measurements. One of our aims is to reveal the most important levels of variation: temporal variation within individual, among-siblings within brood, among broods/spatial variation. One source of variation is dual composition of bird droppings, which consist of white uric acid and darker feces. We collected fresh fecal samples from pied flycatcher (*Ficedula hypoleuca*) nestlings living in a metal polluted area and separated uric acid from feces in order to explore how metal levels (As, Cd, Co, Cu, Ni, Pb) vary between the two matrices. We found that, for all the metals, the levels (d.w.) in the fecal part was more than double of that in the uric acid, more than 2/3 of the total metal load being found in the fecal part. Since the mass proportion of uric acid in the bird droppings varied a lot among samples (mean±SD: 39%±16.9; d.w.), standardizing sampling e.g. by collecting only the fecal part would markedly reduce the variation due to composition. Alternatively, uric acid part can be used as a biomonitor of internally circulated bioavailable metal.

TU250
Lead exposure in Common shelduck (Tadorna tadorna): tracking the success of the Pb shot ban for hunting in wetlands
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Wetlands have accumulated lead (Pb) shot pellets for years due to hunting activities. Lead poisoning in waterfowl due to ingestion of those shot pellets has been well documented worldwide, which led to the ban of Pb ammunition in Ramsar sites and other protected wetlands in Spain in 2001 (Real Decreto 581/2001). However, these Pb shots may persist in the environment for 30-300 years depending on the soil properties and use, remaining available to water birds for long periods of time. Although some studies have evaluated the incidence of Pb shot ingestion in Common shelducks (*Tadorna tadorna*) by exploring the presence of Pb pellets in the gizzard, to the best of our knowledge, no studies have reported Pb concentrations in tissues of this species inhabiting wetlands affected by intense Pb shot hunting in the past.Our main objective was to assess Pb exposure in Common shelducks fourteen years after the implementation of the Real Decreto 581/2001. For this purpose, this study evaluates the Pb shot ingestion in 25 Common shelducks found dead due to a botulism outbreak in La Albufera Natural Park (Valencia, Spain) in 2015, and reports Pb concentrations in internal tissues. Necropsies revealed the presence of steel pellets in the gizzard of five Common shelducks, but no Pb shots were found. The Pb distribution pattern in tissues was bone > kidney > liver > brain > muscle, with Pb concentrations of 778.96±998.64, 122.40±89.18, 92.08±57.65, 17.33±16.46 and 13.32±12.16 ng/g (wet weight), respectively. This study reports for the first time Pb concentrations in internal tissues of Common shelducks in wetlands affected by Pb shot hunting. These concentrations were below those reported in different water bird species inhabiting La Albufera de Valencia before the Pb shot ban. In addition, Pb levels detected were low and below concentrations related to adverse effects in birds.These results suggest that the ban on the use of Pb ammunition over wetlands of international importance has been effective in La Albufera Natural Park. **Acknowledgements**: This study was financed by Fundación Séneca (MASCA-Project 19481/PI/14 and 20031/SF/16).

TU251
Modulatory effects of lead (Pb) in chicken macrophages (HD-11) and B-lymphocytes (DT40) in vitro
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Elevated levels of lead have been found in the environment due to human activities, especially in soil and sediment of historic hunting areas where lead shots were used. Wild birds may ingest spent lead shots by mistake, and after ingestion, lead pellets may dissolve in the intestinal tract and could cause both mortality and various sub-lethal effects, including immunomodulatory effects, for which mechanisms are largely unknown. Once the functions of their immune systems are disturbed by lead exposure, the susceptibility of birds to infections might increase, including avian influenza, which could pose risks not only to birds but also to human populations. To assess the mechanisms leading to immunomodulatory effects of lead, we used two chicken cell lines, chicken macrophage (HD-11) and B-lymphocyte (DT40), as *in vitro* models for different cells of the innate and adaptive immune system in birds respectively. Effects of lead on cell viability were quantified, together with cell membrane integrity, oxidative stress, and several functional endpoints, in both non-activated and activated cells. Cells were activated by poly IC to mimic virus infections, and nitric oxide (NO) production from macrophage, antibody production from B-lymphocytes, proliferation, and expression (qPCR) and secretion of cytokines (ELISA) were the functional endpoints included. Our results indicated that lead was toxic to both cell lines, macrophages being more sensitive. Lead depleted glutathione (GSH) when its *de novo* synthesis by the cells was inhibited, which resulted in increased oxidative stress. NO production in activated macrophages was also increased by lead exposure in a concentration-dependent manner, however, this increase was neutralized by inhibiting the *de novo* synthesis of GSH. The proliferation of both cell lines was also inhibited by lead, especially in non-activated cells. Finally, lead modified the expression of functional immune genes including anti-viral interferons (IFN-α and IFN-β), cytokines (IL-8 and IL-18, etc.) and viral infection related toll-like receptors (TLR3 and TLR7). These results suggest that the adverse effects of lead on the immune system of birds are probably closely related to depletion of GSH, inhibition of proliferation and disruption of cytokines and receptors expressions. However, as the immune system is complex, *in vivo* study will be continued to investigate the long-term and systematic effects of lead exposure on the avian immune system.

Revision of the EFSA Guidance Document on Birds and Mammals (P)

TU252
Challenges in selecting avian reproduction endpoints and options for data interpretation and analyses
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Avian reproduction studies are required to assess the risks to wild birds from potential exposure to plant protection products in many regions (e.g. EU, USA). Avian reproduction studies evaluate the reproductive effects of dietary exposure of adult birds [usually bobwhite quail (*Colinus virginianus*) or mallard duck (*Anas platyrhynchos*)]) to a test substance over a twenty week period, usually performed to OECD (1984) and/or OCSPP (2012). All data and calculated indices of the response variables are analysed to determine statistically significant differences amongst treatment groups. While these animal and resource intensive studies have been routinely performed since the late 1970s, due to the complexity of these studies (high number and nature of response parameters), the limited number of treatment levels (typically three), low number of replicates (12-18 pairs), and high number and nature of the variables assessed, careful data interpretation is required. Statistical differences between the concurrent control and treated groups can arise by chance alone, and can appear to be treatment-related. The aim of this research is to illustrate how different approaches can be used to improve the interpretation of standard laboratory studies by providing further context in the data assessment. The concurrent control should be the most relevant group for comparisons to derive effect levels. However, the historical control data (HCD) from the same (and sometimes multiple) laboratory can also be used to assist in the data analysis. This research examines the interpretation of appropriate statistical analysis for avian studies (which statistical tests for which circumstances), the inherent power of comparisons to determine an effect, the importance of the biological relevance (in relation to the magnitude of effect), and how findings can be applied to the overall reproductive performance of a study in a methodical, comprehensive approach that eliminates the reliance on individual parameters.

TU253
Small mammal male body weight and home range
M. Wang, WSC Scientific GmbH / Dept Efate Modelling; J. Hahne, Bayer AG Crop Science Division; M. Ebeling, Bayer AG Crop Science Division / Ecotoxicology - Terrestrial Vertebrates Expert Team
Longterm risk assessments for wild mammals for plant protection products are usually based on the findings in the laboratory rodent studies conducted for toxicological purposes. Often, it is challenging to identify which of the findings would be relevant for the wild mammal population level. For this purpose, the relevant EFSA guidance includes a schematic view on typical phases of the reproductive cycle, which starts with the establishment of a breeding site and mating. In the EFSA scheme, effects on body weight that are observed in e.g. the rat reproduction study are considered potentially relevant for adverse effects on wild mammals in that phase. As this EFSA guidance is currently under revision, we aimed to evaluate the possible relation between male body weight and home range size for male common voles and wood mice, two species which are typically part of the worst case agricultural risk assessment scenarios. Furthermore, the activity range of both species is very different, so that potentially contrasting results could be expected. Our hypothesis was that larger males might be able to establish a larger activity range and then potentially encounter more females for mating. Since there is no pair bonding between males and females in these species, and both sexes mate promiscuously leading often to mixed paternity litters, a larger female encounter rate might be a key factor for male reproductive success. Thus, if the male activity range were positively correlated with male body weight, then effects on male body weight could be more relevant for the population level risk assessment than if there is no correlation. To our knowledge there is no reference on any European rodent species in which a relation of adult male body weight and home range size has been described or from which such a relation could be concluded. We therefore employed data from 5 yet unpublished field radiotracking studies where individual body weight measurements of common voles or wood mice are available as well as home range measurements were described. These studies were conducted in cereal fields in Germany, either during drilling in spring, drilling in autumn, or in developed fields in late spring. On our poster we present the outcome of this analysis, comparing the home range size against the body weight of male common voles and wood mice. Overall, we found no support for the hypothesis that larger males would have larger activity ranges.

TU254
Revised fruit residue per unit dose (RUD) values for use in wildlife risk assessments of plant protection products
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The EFSA Guidance Document on the Risk Assessment for Birds and Mammals [EFSA GD, 2009] provides default 'Residue-per-Unit-Dose' (RUD) values, expressed as mg a.s./kg fresh weight, for bird and mammal food items to be used in wildlife risk assessments. Some of these default RUDs e.g. monocot and dicot foliage, are based on large numbers of residue studies provided by the industry. However, the RUDs for other diet items, like fruits, were taken from published literature and comprise only small numbers of trials of unclear relevance for regulatory purposes and current EU agricultural conditions. Therefore, many available industry fruit residue studies conducted for regulatory purposes have been evaluated. For this data compilation, the selection of relevant studies providing fruit residue data was based upon the following criteria: GLP-studies conducted in EU during the last 20 years Trials that have been evaluated and accepted as valid by EU member state authorities Trials with one or more applications are considered For the fruit groups 'grapes' & 'large fruits from orchards' (e.g. apple, pear, citrus): Only trials with 1 application are considered For the fruit groups 'other berries' (e.g. currant, raspberry) & 'gourds' (e.g. cucumber, melon)': Studies with one or more than one application are considered and the residue values after the last application taken for the evaluation. Regarding the residue level, this represents a conservative approach (and otherwise values would be based on only a limited number of field trials for these fruit groups) The residue dataset was analysed in terms of the relationship between residue level and application rate. In order to successfully apply the RUD concept given in the current EFSA GD, there should be a linear relationship between the application rate and the resulting residues. The database was then analysed in terms of other 'residue groups' to identify possible differences in residue levels (e.g. depending on fruit size, shape) or differences between regulatory zones. The large dataset of ≥100 residue trials per 'fruit group' revealed significantly lower RUDs compared to the default RUDs given in the current EFSA GD. The authors will present a new proposed default RUD data set for fruits, including some alternative fruit groupings, as diet for birds and mammals that should be considered in the ongoing revision of the EFSA GD. \n

TU255
Comparison of approaches and tool for the determination of time weighted average concentrations using the moving time window approach
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The long-term risk assessment for birds and mammals can be refined considering the substance specific foliar degradation (DT_{50}) and thus, a refined time-weighted average residue concentration. For multiple applications EFSA (2009) proposed to apply the moving time window approach to identify an appropriate MAF × TWA factor. This approach is described in detail in Appendix H of the bird and mammal guidance (EFSA, 2009). Recently, several tools have been developed to facilitate the calculation of the MAF × TWA factor using the moving time window approach. However, existing tools differ regarding their results. In order to facilitate a uniform risk assessment for birds and mammals, differences in the way of calculation and possible deviations from the guidance are identified and an alternative calculation is proposed.

TU256
TREC: Refinement of ecotoxicological risk assessment with a moving time window TWA calculator
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Risk assessment for bird and wild mammals is an integral component of the registration process for plant protection products. In the relevant EFSA guidance document (2009), default values are provided for two parameters relevant for the assessment: MAF (multiple application factor, to account for residue build-up from repeated applications); and 21 day (21-d) f_{TWA} (time weighted average factor, to account for residue dissipation over 3 weeks as default averaging interval). These default MAF and 21-d f_{TWA} values are based on a conservative default DT_{50} values (e.g. 10 days for leaves). The EFSA GD also allows refined calculations of MAF and 21-d f_{TWA} based on more specific DT_{50} values, *e.g.*, from residue dissipation trials. These refined calculations must employ a moving time window approach for 21-d f_{TWA} values. This poster describes the mathematical background and methodology of TREC, the time-weighted average residue calculator developed by the Crop Science division of Bayer for the refinement of risk assessment. Even though the main area of use of TREC is the bird and wild mammal risk assessment, it can be used in any other area of risk assessment requiring calculation of peak exposure or TWA values based on supported kinetic models. The main features of the TWA calculator TREC (implemented as macro-enabled Excel spreadsheet) are: - Full flexibility in definition of the application sequence (e.g. varying application rates and intervals) - Flexibility in the definition of assessment settings (e.g. duration of TWA interval) - Support for various kinetic models - single first order (SFO), hockey stick (HS), double first order in parallel (DFOP), and first order multiple compartments (FOMC) - Possibility to explicitly describe formation of a metabolite from parent - Batch mode for evaluation of multiple trials at once

Fate and Effects of Metals: Advances in Metals Risk Assessment and Regulatory Guidance (P)

TU257
Free indium concentration measured with AGNES: speciation in solution, lability of complexes and dissolution of nanoparticles
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Free metal ion concentrations play a key role in ecotoxicological paradigms such as the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM). Free concentrations of Zn, Cd and Pb have been determined with the electroanalytical technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping)[1-2] in a wide variety of matrices ranging from river water to wine or soil extracts. Other amalgamating elements with negative standard redox potential (such as In, Sb, Sn, Cu, Ga or Tl) are candidates to be analyzed with the same technique. A new AGNES methodology has been implemented for the measurement of free indium, for both, the traditional Hanging Mercury Electrode [3] and the Thin Mercury Film/Rotating Disk Electrode [4]. Speciation of indium in solutions with oxalate and nitrilotriacetic acid indicates that current stability constants in NIST database are not very accurate. The determination of the lability degree of complexes with an ancillary electroanalytical technique allows to optimize the deposition times. AGNES can also be applied to dispersions without any previous separation of the solid or colloidal phases, so it is suitable for providing relevant information about the behaviour of nanoparticles and other colloidal matter, avoiding usual artefacts that can impact on other analytical techniques. The complexation of indium to humic acid has also been studied. The analysis of dispersions of In_2O_3 nanoparticles in background electrolyte and in synthetic seawater will be compared with bulk speciation in contact with solid $In(OH)_3$. The characteristics of the slow dissolution will be discussed. References: 1. Galceran, J., *et al.*, AGNES: a new electroanalytical technique for measuring free metal ion concentration. Journal of Electroanalytical Chemistry, 2004. 566(1): p. 95-109. 2. Companys, E., *et al.*, A review on electrochemical methods for trace metal speciation in environmental media. Current Opinion in Electrochemistry, 2017. 3(1): p. 144-162. 3. Tehrani, M.H., *et al.*, Free indium concentration determined with AGNES. Science of the Total Environment, 2018. 612: p. 269-275. 4. Rotureau, E. *et al.*, Towards improving the electroanalytical speciation analysis of indium. Analytica Chimica Acta, 2018, accepted. The authors from the Universitat de Lleida gratefully acknowledge financial support from the Spanish Ministry MINECO (Project CTM2016-78798). PPV thanks Generalitat de Catalunya for a doctoral FI-AGAUR fellowship.

TU258
Zn availability measured by different analytical techniques
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There is a growing interest in the knowledge of how different chemical species impact on the availability of a given metal. The key role of the free metal concentration is usually highlighted, but the dissociation from complexes (e.g. complexes with particulate matter such as humic acids) or their direct internalization into the roots can also contribute to the uptake from soil. To assess metal speciation, many analytical techniques have been developed, such as those based on passive sampling. The aim of the present work is to apply and compare four techniques to the same Hoagland growth hydroponic media at different concentrations of ligand (EDTA or humic acid) to measure Zn availability. The determination of the free fraction has been performed with AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) [1] and PIM (Polymer Inclusion Membranes) [2] techniques. Labile fractions have been followed with DGT (Diffusive Gradients in Thin films) [3] and LASV (Anodic Stripping Voltammetry with Linear stripping) [4]. PIM and DGT are passive sampling techniques. The information provided by the different techniques will be presented as well as the correlations between the obtained results. A methodology developed for DGT [5], based on the splitting of the flux into a contribution from the free metal ion and another contribution from the complexes, has been applied to interpret fluxes from dynamic techniques, allowing also the determination of the lability degree of the complexes [5]. References: 1. Companys, E., Galceran, J., Pinheiro, J.P., Puy, J., Salaün, P., *Current Opinion in Electrochemistry*, 3 (2017) 144-162. 2. Vera, R., Fontàs, C., Galceran, J., Serra, O., Anticó, E., *Sci. Total Envir.*, 622-623 (2018)

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TU259
HBM (Human Biomonitoring): a tool of environmental toxicology for public health
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Human Biomonitoring is a proven useful tool to assess the exposure to environmental pollutants in general population and a valuable support for improving the development of environmental and health policies to improve the quality of life and the health of citizens. Furthermore, HBM can be apply in the study and follow up at individual level when high exposures are detected. The Spanish Ministry for Food, Agriculture and the Environment funded an HBM program to enhance the understanding of the distribution of priority environmental pollutants in Spain in order to establish reference values for selected heavy metals, persistent organic pollutants and cotinine in the first stage (2007–2010). In order to broaden the knowledge in adolescent population, in 2015 was launched the study BEA (Biomonitoring in adolescents). This is a cross sectional study nationwide in high schools of 10 urban locations. Samples of urine, blood and hair were collected from 500 volunteers of 14-16y. An epidemiological questionnaire to collect basic data, socioeconomic, diet and lifestyles information were completed by the time of sampling. The study was carried out in parallel with that developed by other European countries in the framework of the european project HBM4EU. The work presented is focused on two cases from Huelva and Albacete (7.05 and 13.9 μg/L respectively) that had urinary mercury concentrations above the HBM I-II values defined the German HBM Commission (7 μg/L). Volunteers showing hair mercury levels above the national average were also contacted. The total mercury level of these cases ranged from 5.26 to 7.9 μg Hg/g hair and 71% of them were from Huelva. After the reanalysis of these samples, volunteers were contacted to arrange a new sampling to follow up the levels.A fieldwork team visit the home of the adolescents and collected a new batch of samples including samples from members of the family and performed an exhaustive interview to identify exposure sources of mercury at home. During the home visits and interviews the parents provided greater strength in the answers related to diet, which helped to clarify exposure sources identified in the questionnaires, which were confirmed and additional new ones were identified. The extension of the study at individual level allowed to give specific advices to the volunteers and families to reduce their exposure to mercury. A follow up after receiving the advices is planned to verify the effectiveness of the intervention.

TU260
Delineating sources of mercury and trace metals using epiphytic lichens in Nova Scotia, Canada
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Mercury is a persistent pollutant present in all ecosystems. The prevalence and speciation of mercury will determine its movement in the atmosphere and potential to bioaccumulate and biomagnify through food webs. Monitoring of mercury and other trace metals can be costly, whereas the use of naturally occurring epiphytic lichens can be an effective tool for these types of studies. Nova Scotia, Canada is a hotspot for mercury and other trace metal accumulation in ecosystems, partially attributed to long-range transport of anthropogenic air pollution. The region also contains a number of historic gold mining sites that are known to have persistent high levels of mercury and arsenic in sediment. The relative contribution of local and national sources of mercury to local air is unknown. This work aimed to address which elements can be effectively monitored using lichens. Trace metals in lichens other than mercury may also help elucidate the potential sources of these elements: whether from geological, re-emission, or long-range transport. Almost 200 lichen (*Usnea* spp.) samples were collected across Nova Scotia and analyzed for total mercury (THg); a subset of these samples was analyzed for other trace metals, including arsenic, nickel, copper, cadmium, lead, and selenium (n=95). Significant variation in mercury content was observed across sampling sites and GIS analysis was used to display and model these regional trends. While broad spatial resolution was the initial focus for these collections, a few target areas (biological mercury hotspot Kejimkujik National Park and historic gold mining areas) were sampled more intensively to confirm spatial patterns and if these target areas deviated as expected from background concentrations of mercury and other trace metals. Similar to other monitoring studies of lichens and mosses as bioindicators of mercury deposition, this research provides an excellent baseline for a long-term monitoring program of mercury in Atlantic Canada with ongoing industrial activity and a changing climate. .

TU261
Assessment of air pollution from a shooting range using biomonitors - impacts on physiological and biochemical parameters of lichens
G. Sujetoviene, J. Cesynaite, Vytautas Magnus University
Pollution at shooting range is an issue of growing importance due to accumulation of contaminats derived from ammunition. The aim of the study was to evaluate the changes in buiochemical and physiological parameters and accumulation of heavy metals in the lichens over time transplanted to a shooting range environment. Thalli of the epiphytic lichen *Evernia prunastri* and *Ramalina farinacea* were transplanted from an unpolluted to a shooting range area (central Lithuania). Chlorophyll content, TBARS and the damage to cell membranes in the lichen thalli were determined after 3 month exposure period. The concentrations of some heavy metals (Pb, Cd, Cu) in the biomonitors were used as an indicator of the level of air pollution in the studied area. The accumulated heavy metals in the exposed samples were assessed and the exposed–to–control ratio was calculated in each case. The highest concentrations of heavy metlas were dtermined in thalli near the bern of the shooting range. The presence of pollutants caused the loss of integrity of cell membranes in lichens.

TU262
Mercury in tree bark and lichens at the mining site of Abbadia San Salvatore, Italy: a comparison study
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Biomonitoring is often perceived by the public as more reliable than monitoring accomplished by technical devices. Recent literature indicates that tree barks are, in principle, excellent adsorbents of airborne pollutants, including toxic metals. However, tree tissues still have a relatively small niche when compared to other more widely used substrates, such as mosses and, in particular, lichens. The major disadvantages in the use of lichens are the slow regeneration rates, the irregular and patchy distribution, and the relatively weak tolerance to mycophytotoxic pollutants. Advantages of tree barks as biomonitors are that they are generally widely diffused in many environments, and they do not require a particular botanical training to be recognized and sampled in the field. Disadvantages include a general skepticism concerning the adequacy of barks as reliable bioindicators of atmospheric pollution. To compare the concentration of Hg in barks and lichens in a highly polluted area, we collected *Pinus nigra* J.F. Arnold bark samples from 13 sites in the abandoned Mt. Amiata Hg mining district (Southern Tuscany, Italy; see also the abstract of Rimondi et al., this congress, for further details) and lichen samples from the same trees sampled for barks, or at least from a nearby location. Hg concentrations in lichens (0.18 to 3.57 mg/kg) are essentially similar, at the same location, for the three recognized species (*Flavoparmelia caperata* (L.) Hale, *Parmelia saxatilis* (L.) Ach., and *Xanthoria parietina* (L.) Th.Fr.). The concentrations are of the same order of magnitude of those previously reported for other species in the Monte Amiata area (Bargagli et al., 1989; Loppi et al., 1999), and are one order of magnitude lower than in barks at the same location. The two variables are quite well correlated (R^2 for all species = 0.67), especially for *F. caperata* (R^2 = 0.96). This remarkable agreement between barks and *Flavoparmelia caperata* suggests a similar mechanism of Hg uptake. The overall consistency of Hg distribution depicted by bark indicate that *Pinus nigra* barks can be usefully adopted as well as lichens in environmental monitoring programs.

TU263
Residential Spider web samples as possible bio-indicator of heavy metal polluted environment
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Although spider webs in residential buildings are considered a menace and a possible distortion in the aesthetics values of human environment, there are possibilities that its presence in most residential buildings may be of more benefits than being a pollutant. This study therefore aimed at evaluating the potentials of using cobwebs as possible indicators of heavy metal pollution in residential buildings. Spider web samples were collected from some residential buildings in order to determine the level of trapped heavy metals indoor. These samples were digested in hydrochloric acid and nitric acid before analysis using atomic absorption spectrophotometer (AAS). The result shows that cadmium and lead were present in the cobweb samples while As; Hg and chromium were not present. However, the mean concentration of lead and Cd were found to be significant ($p < 0.05$) when compared with the WHO set standard for residential buildings. Similarly, the concentration of Pb and Cd decreased as the sampling locations were distant away from major roads. The result of this research has shown that spider webs are useful bio-indicator and accumulator of heavy metals

in environment.

TU264
Assessment of Mercury Speciation in Contaminated Tree Bark via Thermal Desorption
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Tree bark of Black Pine (*Pinus nigra* J.F. Arnold) is being used as a biomonitor of atmospheric mercury (Hg) pollution at the historic Abbadia San Salvatore mining district near Mt. Amiata in Southern Tuscany, Italy. Monitoring has shown extreme concentrations of total Hg in bark (as high as 17 mg/kg dry weight) decreasing exponentially with distance away from ore processing areas. But additional work is underway to better understand the speciation of Hg in bark material. Previous work by our research team using X-ray absorption near-edge structure (XANES) spectroscopy showed that Hg in bark samples was dominated by metacinnabar (β-HgS) and Hg-cysteine complexes; Hg bound to tannic acid and Hg^0 were also detected. This poster presents work using an alternative Hg speciation approach: thermal desorption. Using a Milestone DMA-80 analyzer, samples were heated (~8 minutes) and Hg concentrations were assessed incrementally using the following scheme: (1) 175 ºC = labile Hg including $HgCl_2$; (2) 225 ºC = labile organic Hg including humic bound Hg, methyl Hg, and $(CH_3COO)_2Hg$; (3) 325 ºC = insoluble Hg sulfides; (4) 475 ºC = poorly labile Hg compounds including HgO and $HgSO_4$; and (5) 750 ºC = immobile residual Hg. Methyl Hg bark content was also assessed using a warm nitric acid digestion and analysis of liquid digestant via aqueous ethylation, purge/trap, and cold vapor atomic florescence spectrometry. In addition, Hg speciation in limited lichen samples were also assessed via thermal desorption and nitric acid digestion. Testing, which is ongoing, will yield further insight into how Hg is trapped in tree bark, which in turn will help to inform the role that tree bark can play in the biomonitoring of Hg pollution in the environment.

TU265
Making robust decisions in the face of uncertainty in ecological risk management of zinc in Japanese surface waters
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In the ecological risk management, we have to make decisions under many kinds of imperfect information. A framework to make decisions under severe uncertainty is required in chemical management because there are many cases where information about the probability distributions of parameters at the outset is not available. For example, when we set limitations for point sources, there may be an uncertainty on relationship between a chemical concentration in effluent and that in environment, and hence it may be difficult to predict an environmental concentration using the information about effluent. Another example is that the environmental quality criteria are determined to protect the ecosystems, but the criteria may not lead to protect the ecosystems effectively because the ecosystems are complicated. Information-gap decision theory derives the decision that is most robust to uncertainty, by guaranteeing an acceptable outcome under the largest degree of uncertainty. By using information-gap decision theory, we evaluated the best plan to protect the aquatic species against zinc in Kasukawa river as a case study. We have to deal with uncertainties on the following two relationships (1) Environmental concentration and effluent concentration, (2) Adverse effects on the ecosystems and the environmental concentration. We demonstrated the utility of information-gap decision theory in management of pollutant for watershed.

TU266
Bioindication of transport impacts on the forest ecosystem near the main roads
J. Hegrova, V. Jandova, R. Licbinsky, M. Buckova, Transport Research Centre; P. Anděl, Czech University of Life Sciences Prague
Evaluation of the impact of transport on the surrounding environment requires an ecosystem approach and analysing components that provide information about the distribution of the monitored contaminants, and also an estimate of the dynamics of movement and the accumulation of these substances in the environment. As a model ecosystem, a spruce forest with a specific grass and moss undergrowth, located in the area of Bohemian-Moravian Highlands - in an environment which is suitable for a given type of forest, has been chosen. The selected location is located in the immediate vicinity of the highway D1 Prague - Brno in the 103 km highway, before the exposition to Vetrný Jeníkov (in the direction from Prague). This is a locality which is exposed to transport contaminants for a long time. As a result of this survey, the following types of samples were analysed: Soil - soil is the primary component of accumulation of toxic substances in the ecosystem. Samples of fallen needles, humus layer, and first mineral layer were analysed in order to evaluate the distribution of concentrations with the depth and dynamics of the movement of the contaminant in the soil profile. Spruce needles (*Picea abies*) - new, 1-year-old, 2-year-old and 3+ old needles were sampled. The importance of spruce needles as bioindicators lies in the ability to trap toxins in the surface wax layer and at the same time to accumulate substances through the root system and their sensitivity to influence of winter maintenance. Grass (*Avenella flexuosa*) was chosen as a as a model of primary source of toxic substances input into the food chain. Moss (*Pleurozium schreberi*) – was sampled as an accumulation indicator, especially of heavy metals from air pollution. Different types of lichen was sampled because of its different type of matrix (mushroom) as accumulating indicator, especially of heavy metals. To assess the range of contamination, samples were taken at a distance of 5 m from the edge of the road, 20 m and 100 m (as a local background). Transport-related elements show a 40% to 60% higher concentration at the site than in the background (100m). This study was focused on the content of platinum group elements especially in the case of 3+ year old needles, moss and lichen specimens, which are naturally considered as bioaccumulators.

TU267
Does a sum of toxic units exceeding 1.0 imply any ecological effects? A field study of metal impacts on river macroinvertebrate and fish assemblages
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Toxic unit approach based on environmental quality benchmarks (EQBs) such water quality standards and criteria has been used to investigate the quantitative relationship between exposure of chemical mixtures and biological metrics and infer whether there is any ecological risk of concern. Although EQBs are typically conservative (Chapman 2018), it is not conclusive if a sum of toxic units (STU) exceeding one imply any ield effects of concern. To test this question, we conducted a field study in a river receiving a treated mine discharge and a nearby reference river, located northern Japan. At a total of 9 sites including 4 reference ones, we measured several water quality parameters including dissolved concentrations of metals and investigated taxon richness and abundance of macroinvertebrates and fishes. The concentrations of metals (Cu, Zn, Pb, and Cd) at contaminated sites were generally higher than those at reference sites. The STU values based on USEPA water quality criteria (chronic) exceeded 1 at contaminated sites but not at most reference sites. Detailed results on fish and macroinvertebrate metrics are discussed in the presentation.

TU268
The effects of nickel on the structure and functioning of a freshwater plankton community under high dissolved organic carbon conditions: a microcosm experiment
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European environmental risk assessments and environmental quality standard derivation for Ni are currently bioavailability-based. The underlying assumption of this approach is that the bioavailability-based EQS or PNEC derivation, with its associated hazardous concentrations for x% of the species (HCx), is protective for the effects of Ni on natural aquatic communities. In the present study, we aimed to test this assumption by exposing a natural plankton-dominated community to three constant Ni concentrations (a control [no Ni added], and the bioavailability-normalized HC5 and HC50) during a 56d-microcosm experiment under high DOC conditions (dissolved organic carbon 14 mg/L), high pH (pH 8 at test initiation) and low hardness (43 mg CaCO3/L). The protectiveness of the bioavailability-normalised HC5 was evaluated by investigating the effects of Ni on community structure (individual plankton species abundances and community composition) and on community functioning (measured as indirect physicochemical proxies for overnight respiration and carbon fluxes). At the bioavailability-normalised HC5, only short-term effects were observed for two plankton species (i.e. the rotifer '*Trichocerca* group *similis'* and the cyanobacteria *Oscillatoria sp. 1)*, which represent 5% of the species for which the statistical power was high enough to detect significant effects. Community structure and functioning were not significantly affected at the HC5. In contrast, the bioavailability-normalised HC50-treatment had clear effects on both the structure and functioning of the investigated plankton-dominated aquatic community. Yet, long-term effects (i.e. effects occurring over the entire exposure period) of HC50 were only observed for phytoplankton related endpoints. In addition, most of the indirect effects occurring in the plankton community were driven by the direct effect of Ni on the cyanobacteria *Oscillatoria sp. 1*, which was the dominating species in the control microcosms. The long-term No-Observable Effect Concentration for the

investigated community was the Ni HC5, while the long-term Lowest-Observable Effect Concentration was the Ni HC50. Since no long-term effects of Ni were observed at the bioavailability-normalised HC5, our study supports the protectiveness of the bioavailability-based HC5 at high DOC as derived with the bioavailability-normalization procedure that is currently implemented in European legislative frameworks.

TU269

How common carp (Cyprinus carpio) avoid oxidative stress during an exposure to a sublethal tertiary (Cu, Cd and Zn) mixture

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Aquatic environments are subjected to numerous anthropogenic stressors that are much more diverse and variable than those tested in laboratory conditions. So it is important to analyse the effects of metal mixtures to obtain a realistic understanding of the impact of pollution in such natural ecosystems. The impact of a one-week exposure to metal mixture containing copper (Cu), cadmium (Cd) and zinc (Zn) was evaluated in the common carp *Cyprinus carpio*. The concentrations of metals used in this study represent 10% of the 96 h LC_{50} of each single metal exposure (Cu = 4.8 $\mu g.L^{-1}$; Cd = 2.9 $\mu g.L^{-1}$ and Zn = 206.8 $\mu g.L^{-1}$). To evaluate the impact of mixture stress at different levels of oxidative processes and to apprehend their time course within one-week exposure, indicators of oxidative stress (malondialdehyde [MDA] level and xanthine oxidase [XO] activity), as well as activities and gene expression of key enzymes involved in antioxidant defense (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx], glutathione reductase [GR] and glutathione-S-transferase [GST]) were measured in gills and liver of *C. carpio*. In addition, the total antioxidative capacity (TAC) was quantified. No sign of oxidative stress was observed during the experiment, but CAT, GPx, GR and GST activities were significantly reduced in the liver after 7 days of exposure. This suggests a potential decrease of glutathione levels and risk of increased free radicals in case of longer exposure. In the gills, there were no major changes in the TAC or in the activities of antioxidant enzymes but the relative expression of the genes coding for CAT and GR were triggered, suggesting an effect on the transcription processes. These results prove the high tolerance of *C. carpio* to these levels of metal pollution, at least in this short-term exposure.

TU270

Toxicogenomic analysis of zebrafish responses to complex environmental metal mixtures

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The increased use of metals in different applications has become a global environmental health concern. This has led to an urgent need to improve the tools to determine the effects and consequences of exposure to different metals, alone or in combinations. Environmental risk assessment is often based on, chemically measured, total metal levels without considering bioavailability, speciation or matrix effects. As most of the metals released into the environment eventually end up in water, aquatic organisms are at a higher risk of exposure to these metals. In the present study, we exposed zebrafish from 0 days post fertilization (dpf) to 5 dpf, from 0 dpf to 8 dpf and from 15 dpf to 21 dpf to water collected from different Swedish mining sites to study survival, hatching and gene expression patterns. As an indicator of physiological responses the expression of genes representing toxicity, reproduction, cancer and immune system responses were analyzed. The qRT-PCR analysis were conducted by using 3 different controls, a site specific control (upstream from each site), an general environmental control (a clean site) and a laboratory control (zebrafish aquarium water) to determine the influence of control selection on the outcome of environmental risk assessments. The results showed that gene expression analysis was more sensitive than the determination of physiological endpoints. In order to obtain results that are relevant from a site-specific point-of view it is important to include controls from the studied environment as this minimizes differences in bioavailability, speciation and influence of other abiotic factors. Comparing site specific controls to the laboratory control and the general environmental control, indicate that the use of general controls will result in effects that are not related to the exposure conditions. Thus, site specific analysis should be included when performing environmental risk assessment. In addition, this study supports the use of toxicogenomic analysis to correlate exposure to effect and thus should be of great value in environmental risk assessments. Keywords: Metal, gene expression, site specific, environmental risk assessment

TU271

Toxicokinetics of metals in Enchytraeus crypticus: towards an Adverse Outcome Pathway

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The Adverse Outcome Pathway (AOP) concept was proposed as a tool for chemical risk assessment in 2010. This framework aims at causal understanding of biological responses on different levels of organismal complexity, from the molecular up to the apical phenotypic or population effect levels. The determination of the molecular initiating events within an AOP is challenging, and depends on various factors, e.g. chemical-biomolecule interactions, exposure concentrations and also on toxicokinetics and toxicodynamics. Assessing toxicokinetics is helps understanding the linkage between external and internal exposure, which in fact is the first step in an AOP, the chemical-biological interaction. The aim of the study is to investigate the toxicokinetics of a number of selected metals using the soil invertebrate *Enchytraeus crypticus* (Oligochaeta, Enchytraeidae). Bioaccumulation tests were performed in which the animals were exposed to soil spiked with $CuCl_2$, $CdCl_2$ and $CrCl_3$. Exposure consisted of 14 days for uptake followed by 14 days in clean soil. During this time, animal and soil samples were collected at several sampling times, following the recommendations of OECD guideline no. 317. The animals were analyzed for Cu, Cd or Cr body concentrations by graphite furnace atomic absorption spectrometry. Body concentrations will be related to total and available metal concentrations in the soil. The results will enable a better understanding of the time point at which the chemical reaches its initial target molecule, helping addressing the question of molecular initiating events, for which the toxicokinetic behavior of a chemical is essential. Currently, chemical analysis of animal, soil and pore-water samples are ongoing, after which data analysis will follow. This is part of a larger project aiming to fill in information at additional levels towards an understanding of the mechanisms and with a strong focus on the time series sampling and observations, a current gap.

TU272

Identification of thermal effect on cadmium toxicity toward Daphnia magna

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In aquatic environments, organisms are exposed to combinations of stressors and their effects have been demonstrated to be interactive. Considering that elevated temperature (natural stressor) tends to increase metal toxicity (chemical stressor) to aquatic organism, the comprehensive understanding of this interactive effect is particularly important. Therefore, the aim of our study was to identify the thermal effect on cadmium (Cd) toxicity by experimentally evaluating life-history traits of *Daphnia magna* at different conditions of temperature (20 or 25 °C) and Cd concentrations (1.0, 2.5, 5.0, 7.5, 10 µg/L). Our results indicated that chronic Cd toxicity to *D. magna* was higher at 25 °C than at 20 °C, in particular, with increasing of male production. At 25 °C and the highest Cd concentration (10 µg/L), reproduction was 63% lower and sex ratio was 44% higher than those at 20 °C without Cd. Additionally, synergistic effects were found in population growth rate for high Cd concentration groups (7.5 and 10 µg/L) at elevated temperature. Overall, this study suggests the importance of studying interaction among multiple stressors to interpret their adverse effect on aquatic organisms adequately.

TU273

Ecotoxicology of Rare Earth Elements (REE) in continental aquatic systems: biological effects and speciation

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Rare Earth Elements (REE) became essential in high- and green-technology applications. Their increasing production and use have led to the release of anthropogenic REE in the environment, including aquatic systems. However, little is known about the ecotoxicology of REE, particularly in complex systems, preventing the implementation of regulatory thresholds. To understand better the behaviour and biological effects of REE, we conducted acute ecotoxicological assays on several freshwater species belonging to different food web levels: the green microalga *Raphidocelis subcapitata* (ISO 8692, 2012), the crustacean *Daphnia magna* (ISO 6341, 2012) and the fish *Danio rerio* (OCDE 203, 1992). We test three elements representative of the REE group: neodymium (Nd), gadolinium (Gd) and ytterbium (Yb) alone and in mixture. The assays were performed in absence and presence of dissolved organic matter (DOM: 8 mg/L of dissolved organic carbon including 6.8 mg/L fulvic acid). First results show comparable effective concentrations (ECx) for Nd, Gd and Yb, which suggests a similar toxicity pattern among all REE. The presence of DOM tended to decrease the toxicity of REE. These results should be considered in environmental risk assessment.

TU274

Unicellular green algae show great variation to uranium toxicity in boreal freshwaters

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Uranium (U) is a naturally occurring element that can be toxic because of its radioactivity and its chemical properties. Due to mining activities it can cause contamination in effluent-receiving freshwaters. There its chemical toxicity is the major cause for concern rather than its radiation toxicity due to its low specific activity, which is a result of the long half-life of U-238. Boreal freshwaters are often rich in dissolved organic material (DOM) that can bind metals such as U, leading to lowered bioavailability. There is limited information on the chemotoxicity of U to algae, especially in boreal freshwaters containing DOM. We studied the effect of U on the 72 h growth of two freshwater algae *Raphidocelis subcapitata* and *Desmodesmus subspicatus* according to a slightly modified standard protocol by ISO 8692, using boreal lake waters with a range of 4---18 mg/l dissolved organic carbon (DOC) as test waters. Whereas the 72 h NOEC value for U in both species was similar in the water with the highest DOC concentration, in other freshwaters *D. subspicatus* was less sensitive to U, with up to ten times higher NOEC values and up to 14 times larger IC_{50} values. In both species, there was a clear trend of diminishing toxicity of U with increasing DOC concentration.

TU275

The effects of Se in Limnodrilus hoffmeisteri and Oreochromis niloticus

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Selenium (Se) is beneficial at low levels yet toxic at high levels. The present study assessed the bioaccumulation and effects of different Se species from the dietary exposure route in two aquatic organisms: the oligachate *Limnodrilus hoffmeisteri* and tilapia *Oreochromis niloticus.* The worms were exposed to 2, 5, 20, and 40 μg/g sedimental Se in the form of Se(IV) and Se(VI) for 2 months. The tilapia were exposed to dietary Se in the form of Se(IV) and Se-yeast for 90 d. The accumulation of Se in both species followed a general pattern of organic Se > Se(IV) > Se(VI), indicating a higher risk of organic Se than inorganic Se at the same exposure levels. In the worm experiment, its growth was inhibited at Se(IV) levels higher than 2 μg/g while it was inhibited at Se(VI) higher than 20 μg/g. The subcellular fractionation showed that Se existed predominantly in cell debris for both Se forms. The antioxidant system was affected differently after 2 weeks and 2 months of exposure, indicating adaption of this worm to Se during chronic exposure. In the tilapia experiment, the inhibitory effect of Se on the weight of tilapia was only observed at Se level of 12 μg/g dry wt. The accumulation of Se in various tissues of tilapia (e.g., liver, intestine, brain, muscle) was generally dose dependent. The levels of MDA were more elevated after 45 d of exposure than those after 90 d of exposure, indicating certain degree of adaptation to Se. Transcriptional expression of genes related to immunological function showed that both Se species enhanced the immunological factors at the levels 3, 6, 12 μg/g. The results showed that Se accumulation in the two species from the dietary source differed. Dietary Se showed both beneficial and detrimental effects in aquatic organisms, dependent on Se species and exposure duration.

TU276

Acute toxicity of different toxicants to Freshwater Pearl Mussel juveniles (Margaritifera margaritifera)

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Margaritifera margaritifera is one of the most endangered mollusk species in Europe. The population of this freshwater pearl mussel has been declining since the 20th century due to pearl fishing, alteration of the water quality, habitat degradation, decline of the fish host or pollution. Several programs have been set up in order to preserve this species by restoring habitats and by the application of captive breeding techniques. However, there's a lack of knowledge on the sensitivity of this species to environmental and contamination factors. As part of the European LIFE Project (LIFE 13 NAT/FR/000506) which aims to preserve the most important French population of *Margaritifera margaritifera* (Dronne River – Dordogne), a part of the production from captive breeding facility is used for ecotoxicological studies. This work deals with the acute toxicity of Sodium Chloride (NaCl), Nitrates (NO_3^-), Phosphates (PO_4^{3-}), Cadmium (Cd) and Arsenic (As) to juveniles of this species. Acute toxicity tests were carried out on 10 to 21 months old juveniles in order to assess the toxicity thresholds of those toxicants and to compare the sensibility according to age. First, the experimental protocol has been developed using Sodium Chloride as reference toxicant. After that, the use of a substrate (silicate sand) for experiments has been studied compared to conditions without any as preconized by the American Society for Testing and Materials (ASTM) *Standard guide for conducting laboratory toxicity tests with freshwater mussels*. The toxicity threshold obtained for NaCl reveals a greater sensibility of *M. margaritifera* juveniles than for some other species of freshwater mussels. Also, results showed that juveniles of *M. margaritifera* aged from 10 to 21 months were tolerant to high concentrations of NO_3^-, PO_4^{3-}, Cd and As. Moreover, the comparison of sensibility in function of age reveals that younger juveniles were more sensitive than older individuals. The use of sand for acute toxicity tests has no impact on water quality, toxicants concentrations and results, validating our method of exposure in more realistic conditions for burrowing mussels. This study allowed us to improve our knowledge about this endangered species and showed that ecotoxicological studies could be a complementary approach for helping conservation strategies since it gives us useful data for targeting reintroduction areas.

TU277

Modeling dietary uptake of heavy metals in stream macroinvertebrates of different feeding classes and their food

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Streams serve a number of vital functions in ecosystems. In developed areas, metals along with other stressors impair these functions. The aquatic life criteria, established by the US-EPA, is one of the current practices of identifying stream quality. However, it lacks representation of a family of organisms-insects. Tools such as biotic ligand model can be used to modify acute concentration for some metals such as Cu. However, dietary uptake of different metals and differences in metal speciation or long-term exposure has always been unaccounted for in established toxicity assays. Hence, to protect stream health in chronic exposure cases, establishing different benchmarks and alternative means of assessment is necessary as opposed to setting daily average contaminant concentration limits. This requires a better understanding of the speciation of metals from different sources and their impact on biouptake. Dietary uptake is known to be an important exposure pathway for macroinvertebrates, differences in uptake and assimilation of metals as a function of the unique source waters in urban stream systems are unknown. This study aimed at modeling the differences in uptake and assimilation from water borne and diet borne pathways for different selected stream organisms representing various feeding classes exposed to various contaminated source water. Uptake of metals into the food, periphyton, was investigated in exposure experiments with ^{63}Cu and ^{65}Cu isotopes rendering ~5 mg/L total copper. Collected stream water, wastewater treatment plant (WWTP) effluent and their mixture was used as sourcewater. To model uptake into the macroinvertebrates, the organisms and their food will be exposed to exposure water reconstituted with concentrated organic matter from the source waters and metal isotopes. Results show uptake rate (6-17 mg $g^{-1}h^{-1}$) into periphyton to be higher for source water with high Cu/DOC. Metal speciation related to higher uptake rates and high percentage of copper weakly bound to OM. The exchangeable fraction was established as the bioavailable fraction by analyzing conditional uptake rate constants. Long exposure experiments show uptake equilibrium after 2 days with a maximum accumulation of 40 mg/g. Switching isotopes after this period is planned to calculate efflux rate. Results from planned experiments are expected to yield relationships between source water metal speciation and OM characteristics and net assimilation into the macroinvertebrates.

TU278

The fate of metal ions in aquatic systems: Implications for environmental hazard assessment of inorganic substances

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The assessment of long-term environmental hazard of chemicals includes consideration of their environmental fate. This is described in terms of degradability – reflecting the reduced potential for long-term exposure of organic chemicals which are quickly degraded in the environment. While the concept of degradability has limited meaning for metals and inorganic chemicals, the fate of such substances in aquatic systems may equally be affected by natural removal processes which reduce the potential for long-term exposure. Ample laboratory, mesocosm, and field data confirm this for many different metal ions. The relevant processes include binding of metal ions to suspended particles, settling and burial of particle-bound metals, chemical transformations due to ageing reactions or redox changes, and others. Clearly, there is a need for guidance to implement this concept in the hazard classification of metals and inorganic substances. The work presented here describes the development and application of a standardized test method to evaluate the removal of metal ions from aquatic systems. The existing Transformation-Dissolution protocol (OECD 29) was extended with 2 phases: a removal phase, to measure the rate and extent of metal binding to a substrate and subsequent settling, and a resuspension phase, to address the potential for metal remobilization. A small amount of substrate is added to the aquatic test medium to initiate the partitioning and removal processes. The Extended Transformation-Dissolution protocol was successfully applied to several metal ions, including copper, nickel, lead, zinc, cobalt, silver, and strontium. Mechanistic modelling showed that the measured removal rates are mainly determined by metal ion diffusion to settled particles and by the kinetics of metal binding to particles. An evaluation of the sensitivity of the test results to the most important system defaults was performed. Overall, the Extended Transformation-Dissolution Protocol is a promising tool to assess the fate of metal ions under standardized conditions, and could allow recognition of the fate of inorganic substances in environmental hazard assessment.

TU279

Probabilistic risk assessment for freshwater tilapia species exposure to mercury

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Mercury (Hg) is a highly toxic, globally ubiquitous contaminant that can be seriously damage environment and human health. A growing body of evidence has indicated that inorganic Hg (Hg(II)) and methylmercury (MeHg) exposures have elevated in Taiwan major rivers. Moreover, Hg contamination is highly likely to pose a threat for freshwater fish decline in populations. As a result, Hg exposures are intensively investigated by Environmental Protection Administration of Taiwan (Taiwan EPA) including surface water, sediment, and fish tissue measurements. Here we established the key risk indices—hazardous quotient (HQ) and exceedance risk (ER)—for freshwater tilapia species across geographic ranges of several major rivers in Taiwan based on a probabilistic risk assessment framework. This study took account three tissue burden-specific mortality rates to quantify mortality risks of tilapia exposed to Hg(II) and MeHg. Our results indicated that there was 50% probability for mortality risk of tilapia to exceed ~5% by considering three tissue burdens of gill, liver, and muscle. Noticeably, even if we considered the worst scenario of the highest accumulated internal dose in liver (ER = 0.2, i.e., the risk probability that might be unlikely to occur), mortality rate of tilapia would not exceed 5.4%, indicating low mortality risk for tilapia exposed to Hg(II) and MeHg. We further chose three safety levels to examine the potential hazard to the organisms in aquatic environment, i.e., 0.2, 0.4, and 0.5 $\mu g\ g^{-1}$ ww in whole body of tilapia. Our results indicated that with the stricter safety levels being adopted, the higher hazard would be presenting. In northern region of Taiwan, the median values of HQ in three rivers would be over 1 based on the strictest safety level. Thus, a dramatically high HQ was found in our risk assessment outcomes, implicating that the chronic sublethal risk (e.g., growth, reproduction, development, and behavior) posed by Hg was alarming. We conclude that Hg is likely to pose the potential hazard to aquatic environments constrained by safety levels for aquatic organisms. Our approach opens up new possibilities for predicting future fish population health with the impacts of continued Hg exposure to provide information on which fish are deemed safe for human consumption.

TU280

Use of monitoring data for environmental risk assessment of copper in soil at the European scale

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The diffuse contamination of soils with copper due to various sources (e.g. sewage sludge, manure, fungicides, releases from brake pads, etc.) can cause a significant accumulation of Cu in soils. A proper risk assessment of Cu in soil not only requires data on Cu concentrations, but also on basic soil physico-chemistry data (pH, organic carbon, clay content effective cation exchange capacity) to predict the bioavailability and hazard of Cu in the specific soils monitored. The spatial variation in metal bioavailability and in exposure concentrations is preferentially considered in risk assessments at the regional scale. Data availability for both aspects differs largely across different countries or regions, hampering a consistent approach on a large (e.g. regional or continental) scale. It is therefore often difficult to compare results for regional risk assessments among different countries when based on national datasets. Fortunately, two European–wide monitoring datasets were developed the last decade. The LUCAS (Land Use/Land Cover Area Frame Survey) project from the European Joint research Centre (JRC) provides data for soil properties and land use for about 20,000 soil samples covering the EU. All data were collected according to harmonized sampling and analysis methodologies. In addition, also the GEMAS (geochemical mapping of agricultural and grazing land soil) project, carried out by the EuroGeoSurveys (EGS) Geochemistry Expert Group in cooperation with Eurometaux, provides exposure data of metals and general soil properties in agricultural and grazing land soil at the European scale, harmonized with respect to the spatial scale (sampling density), analytical methodology and land-use (comparable level of diffuse emissions). The results of both projects allow a consistent hazard and risk characterisation for Cu in soils across Europe and country-specific results can be directly compared. Because all data are geo-referenced, the data also provide a strong basis for considering the spatial variability of both exposure and effect concentrations (through variation in soil properties and bioavailability corrections) in a risk assessment for Cu in soils and therefore avoid the need for (worst-case) assumptions on both aspects. This poster will present and discuss the use of these monitoring data for regional and local risk assessments of copper in soil.

TU281

Integrated environmental impact assessment of old mining activities along the Upper Rhine

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In mining areas, both anthropogenic disturbance of biogeochemical cycles of metallic elements and natural weathering of rock materials rich in these elements could lead to high metal concentrations in the environment. River ecosystems draining old or recent mining areas could present metallic trace element (MTE) concentrations high enough to induce toxic effects to biota. In France (Vosges) and in Germany (Black Forest), mining of silver-bearing mineralization has generated punctual high ETM pollution as arsenic and lead in downstream rivers. In order to assess the potential environmental impact of old mining activities and toxicity of ETM under more environmental realistic conditions, our strategy is to propose an interdisciplinary approach including experimental works and modeling to characterize and assess: The quality of water and sediments from several mining sites The eco-dynamics of metals from mine areas to aquatic ecosystems using passive sensors and living organisms The ecotoxicity of metals on target living organisms and communities (micro-organisms, benthic invertebrates, macrophytes) The potential trophic transfer of ETM in the aquatic food web The effects of ETM on the ecosystem functioning The historical use of wood resources for industrial/mining activities and impacts on forest ecosystem For this integrated environmental approach, we will use pre-existing databases, historical written sources, such as historical management plans and historical maps, phyto-historical indicators from charcoal production sites as well as physicochemical measurements, next generation sequencing technology to describe adapted microbial communities, passive and active biomonitoring with macrophytes and benthic invertebrates and ecotoxicological and functional biomarkers and bioassays. First results of the phyto-historical approach, based on the anthracological analysis of seven charcoal production sites in two different areas indicate that the most used trees were *Fagus* and *Abies*. Other, less present taxa, were *Acer, Betula, Quercus, Sorbus,* and *Pinus*. The radiocarbon datings of the charcoal production sites of one of the two investigated areas show an activity of charcoal production during the 18th century. These first results are coherent with comparable regional data about the history of charcoal production. More analyses are planned on other charcoal production sites and with dendro-anthracological measurements.

TU282

Probabilistic and site-specific risk assessment of heavy metals in Korean Rivers

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Water regulations for heavy metals in South Korea have been designated for human health (e.g., regulation for the protection of human health) and usages (e.g., regulations for drinking water, agricultural water, and effluent water). However, these regulations are considered to be unsuitable for the protection of aquatic ecosystems because many organisms in water are critically affected by low levels of heavy metals. This study constructed a species sensitivity distribution (SSD) to scientifically derive thresholds for protecting organisms from heavy metals (Cd, Cu, Pb, and Zn) in Korean freshwater. The SSD has been developed by integrating individual toxicity value of various organisms and assigning the proportion among the community or population and has been agreed its scientific relevance with many researchers and jurisdictions. The toxicity values were composed of the acute response of Korean resident species and standard test species, and toxicity variability caused by different water quality was adjusted by hardness conversion. The critical thresholds were derived from predicted no-effect concentrations (PNECs) which only few organisms in the SSD are expected to be significantly affected. The ecological risk of freshwater heavy metals in Korea was investigated by comparing the PNECs derived from resident species and environmental concentrations detected in the four major rivers of Korea. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through "The Chemical Accident Prevention Technology Development Project", funded by Korea Ministry of Environment (MOE) (No. 2016001970001).

TU283

Risk and biological assessment of heavy metals in the River Aconcagua, Chile

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The long-term exposure to heavy metals may reduce natural diversity and change the genetic variability of aquatic organisms. These alterations may end up affecting population fitness and making impacted aquatic organisms more sensitive to new and future stressors. Usually, environmental risk assessment frameworks for heavy metals are based on metal-by-metal assessment, but metals are most often found as complex mixtures in the environment. Chile has the largest copper mining activities in the world and the River Aconcagua is one of the most important rivers in central Chile. Extensive heavy metal data is available for this river; however, little is known about metal mixture ecotoxicity and the effects that this may have on local aquatic populations. This study addressed these knowledge gaps, with the main objective to investigate whether complex metal mixtures affect the biodiversity patterns of freshwater organisms along the River Aconcagua. For this purpose, the metal status of the Aconcagua freshwater ecosystem was described in detail and the biological status of microbial and macroinvertebrates communities along the river was defined using both next-generation sequencing techniques and taxonomic classification. Furthermore, an environmental risk evaluation of metal mixtures under the EU Water Framework Directive using ecotoxicological available information was performed and concentration addition and independent action approaches per each ecological level (algae, invertebrate, and fish) were applied.

TU284
Evaluating the potential of biochar and compost as a growth layer for the cover system of mine tailings in northern Finland
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Mine tailings represent a source of toxic pollutants, mainly heavy metals, which may spread to the surrounding areas. After ore-extracting activities mine tailings need landscape reclamation to prevent environmental risks. Recyclable organic waste materials e.g. compost and biochar are nowadays progressively used for soil improvement and carbon sequestration in horticulture and landscaping. Recently, biochar and compost have also been suggested to be used in closed mine tailings to enhance their phytostabilization and being a long-term and cost-effective rehabilitation strategy to reduce the risk of pollutant transfer. However, information about the feasibility of those materials as a growth medium layer in mine tailings and their effects on the vegetation restoration, reforestation success and leaching of pollutants is scarce, especially in the boreal climatic conditions. We examined the physical and chemical characteristics of two biochar types (spruce and birch), composted sewage sludge (CSS), tailings soil and forest till soil as well as their effects in varying mixing proportions on plant growth (Scots pine, dark leaved willow, red clover, red fescue and maiden pink) during one growing period in a greenhouse study. In addition, we established the large scale lysimeter study in the Rautuvaara mine tailings site in Kolari commune using three kinds of cover layers: 1) forest till soil, 2) till soil added with CSS (10%) and 3) till soil + CSS (10 %) + biochar (of Norway spruce) (10 %). Effects of various cover layers on i) the properties of leachate water (pH, oxygen content, moisture, redox potential, metal and nutrient concentrations) and ii) vegetation success (growth, covering area, clorofyll content, concentrations of metals and nutrients) will be investigated during two growth periods.

TU285
Evaluation of landfill impacts on mountain rivers
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Physico-chemical measurements provide limited information in the environmental quality assessment of mountain rivers. Indeed, these systems have high flow rates, often artificially controlled by dams, and river beds with low quantities of coarse sediment. These characteristics make challenging to trace diffuse pollution, such as those represented by waste landfills scattered along rivers. Analysis performed on inhabiting organisms to complement these measures are also known to be rather limited in such environment due their high variability. Here, we examined the usefulness of metal accumulation measurements in bioindicators and effects on model organisms to evaluate the bioavailability of metals released from mineral processing landfills. The two studied rivers situated in the French Alps were sampled up- and downstream of landfills, four times during one year, for water, sediments, biofilms, bryophytes, and fish (*Salmo trutta fario*). Water was analysed for its physico-chemical parameters (pH, concentrations of dissolved organic carbon, anions, cations, metals) whereas metal speciation was calculated using Visual Minteq. Sediments were analysed for their metal concentrations and composition. Metal concentrations were measured in both biofilms and bryophytes (total and intracellular) as well as in fish (in gills, muscle, gonads and liver). Ecotoxicological tests were finally performed on water (algae and daphnids) and sediment (ostracods and plants). Water analysis highlighted seasonal increase of Co, Ni, and As concentrations between the control site and those located downstream the landfills in one of the studied river. That increase was accompanied by an increase of Co, Ni and As accumulation in biofilms and bryophytes as well as of As and Hg in fish tissues. However, no direct correlation could be drawn between the free metal ion concentrations and the metal accumulated in biofilms and bryophytes. For both rivers, no difference in sediment metal concentrations was observed between up- and downstream sites, which could be explained by their sediment composition containing *ca*. 90% of coarse sand and a low percentage of organic matter (0.5 – 2.0%). Ecotoxicological tests did not reveal any effects of the water quality on model organisms but demonstrated degradation of sediment quality. This study is an attempt to provide insights in comparing and reconciling ecotoxicology laboratory tests and field weight of evidences for metals impact assessment.

TU286
Barrage fishponds: fitted to reduce pesticide concentrations in downstream rivers, but ineffective against trace metal pollution
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In France, fish ponds cover up to 3500 km², but because of their small size, they have been little studied and their ecological functioning as well as their impact on water quality are poorly known. Frequently located at the head of watersheds, their impact on water quality is of first importance for the water bodies located downstream and monitored under the Water Framework Directive (WFD). Ponds also represent ecosystems of great interest, both environmentally (biodiversity hotspot) and economically (extensive fish production). Studies carried in our labs show that the impacts of these water bodies, on downstream systems, depend on fishpond management and can be either, punctually a source of contaminants (e.g. during the draining of ponds), or on average, an efficient buffer for pesticides. Our previous study carried on three dam ponds have demonstrated a reduction of pesticide concentration peaks as well as a reduction in potentially deleterious effects for downstream ecosystems. We wanted to evaluate whether these ponds could also be an effective remediation tool for another contaminant monitored under the WFD, namely trace metals. The experimental set-up used to quantify pesticides fluxes on dam ponds also made it possible to follow the annual evolution of the upstream / downstream flows of certain metals of interest. The concentrations of cadmium, lead, zinc and copper were quantified in the dissolved phase upstream and downstream of the studied ponds. For metals, no significant reduction was observed. In two ponds, it is even a trend towards increasing concentrations downstream that is observed and not a decrease, suggesting direct cross-sectional contributions from the watershed (e.g. runoff from adjacent crops), or even intra-pond remobilization. Contrary to what was observed for pesticide flows, fish ponds do not appear to induce retention of trace metals. Given the importance and diversity of sources emitting trace metals (e.g. phosphate fertilizers, metallurgy, military munitions from world wars), it seems difficult to determine a single origin in the sampled surface waters. Ponds play a beneficial buffer role on pesticide pollution, by reducing concentrations in streams, they mitigate the risk of acute and chronic toxicology related to these contaminants, particularly in headwater streams, but they are inefficient regarding trace metal pollution.

TU287
Cost-effective mitigation of lead toxicity in rat using indigenous plant Moringa Oleifera
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Health and environmental issues caused by lead (Pb) are commonly recognized as a global challenge. Pb possesses particularly elevated anthropogenic enrichment factors which are important for the nation's development especially in developing countries. To enjoy the economic benefit given by industrial activities to the fullest extent, it is needed to establish a cost-effective technique to mitigate pollution and poisoning towards the practical use in developing countries. *Moringa Oleifera* (MO) is a multipurpose plant widely distributed in many countries of the tropic and subtropics. Previous researches suggested that the leaves and seeds of MO have a potential to mitigate Pb toxicity on rats. Similarly, it is commonly recognized that the seed of MO can be useful for the removal of metals from polluted water due to its coagulating properties. From above, MO is considered as the potential plants for both medical use for human and remediation purpose of environmental contamination. Given these backgrounds, the current study aims to evaluate the mitigative effect of MO on Pb poisoning in rat. All animal experiments were carried out under the supervision and with the approval of the Institutional Animal Care and Use Committee of Hokkaido University (approval No. 18-0042). Fifty male SD rats were divided into eight groups (N=7 for group A and B, N=6 for group C to H). 1,000 mg/L of Pb acetate were given to all groups other than group A in the drinking water for 3 weeks. The control

group A were provided with distilled water. Two different doses of MO leaves: 100 and 600 mg/kg-body weight were administrated orally using zondes to group C and D, respectively for 3 weeks. Group E and F were given the water extract of MO leaves with doses of 100 and 600 mg/kg, respectively. Group G and H were exposed to the MO seeds powder of 100 and 600 mg/kg, respectively. After exposure, rats were sacrificed for the collection of blood and organ samples. Metal analyses and blood biochemical test were performed. No significant differences were found in Pb levels in the blood, liver and kidney of all groups except for control group, whereas the mitigated trends of Pb toxicity on albumin and creatine level were discovered. Further analysis will be done to verify the effect of MO on Pb toxicity and described at the presentation. Additionally, the perspectives of another study looking at the possibility of MO for environmental remediation purpose will also be presented.

TU288

The trend of elevated blood lead levels in Kabwe mining area, Republic of Zambia

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Lead (Pb) possesses a particularly elevated anthropogenic enrichment factor and is a toxic metal for organism. Although Pb poisoning is preventable, it accounts for approximately 0.6% of the global burden of disease, with the highest burden in developing countries. Namely, it's one of the most common and best-recognized childhood diseases of toxic environmental origin. The town of Kabwe, a central provincial capital of the Republic of Zambia has a long history of Pb and zinc (Zn) mining which operated for nearly a century without proper laws regulating emissions from the mine. There are many activities going on such as metal scraps scavenging from the abandoned tailings, use of Pb-laced soil to make bricks and agricultural activity at the contaminated area around mining site. It is considered that these processes and dust emanating from the mine dumps have continued to serve as a source of metal pollution. Our former researches have been revealed the serious contamination status in Kabwe with findings of high levels of Pb accumulation in the environmental soil sample, wild rats, livestock, poultry and even children. The current study was conducted to assess the prevalence of blood lead levels (BLLs) in the general population of Kabwe, and to compare the prevalence of BLLs within the region and with other studies worldwide. Thus, the blood sample was collected from Kabwe residents at various clinics around and far from the mining area to clarify how far Pb diffuses and makes an impact on human health. The BLLs were tested using LeadCare® II analyzer. In total, blood samples were collected from 1270 participants in 11 clinics and BLLs ranged from LOD to 162 mg/dL. High BLLs were observed from people living near to the mining site and most of them had BLLs exceeding the CDC reference value of 5 mg/dL that raises "health concern". Generally, BLLs in the vicinity of the mining area were significantly higher than those in remote sites ($p < 0.01$). In summary, the present study has demonstrated Pb poisoning in Kabwe residents, especially in areas close to the mining site. Further toxicological analysis is essential to evaluate the exact generated toxic effect in the body. Also, regular monitoring and proper countermeasure should be taken immediately.

TU289

Source Identification and Metal Analysis of Fine Particulate Matter (PM2.5) in an Industrialized Urban Area of Lagos State, Nigeria

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Gaseous pollutants and particulate matter are released into the atmosphere at concentrations above their normal ambient level; this is caused by the increasing human activities which eventually have a measurable effect on humans, animals and plants. Particulate matter (PM) which can be inhaled into the human respiratory system is related to most serious health effect including pulmonary and cardiovascular illness. Based on the field study conducted, the concentrations of PM2.5 at the different locations vary with respect to anthropogenic activities. The PM2.5 levels obtained ranged from 14.00 to 32.67 µg/m3during wet seasons and 18.67 to 34.67 µg/m3 during dry season. Trace elements especially heavy metals are significant components of PM2.5 in industrial environments. The heavy metals are of particular concern due to their persistence in the environmental media and their human toxicity.The particulate matter concentration was obtained using Casella cel-712 microdust pro-real time dust monitor and flame atomic absorption spectrophotometer was used to determine the elemental content. The Principal Component Analysis explained three common contributing sources of fine particulates ($PM_{2.5}$) such as entrained soil, sea salt and combustion. Correlation matrix was also determined and some of the elements were strongly correlated while some were not.

TU290

Metal accumulation in two crops across the Mbale Waste Dumpsite

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Over 800 million people globally practice open dump farming, and this could pose health risks. This study investigated the occurrence, spatial variations and threats to food safety of aluminium (Al), chromium (Cr), iron (Fe), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), mercury (Hg), and lead (Pb) in *Zeamays* and *Amaranthus cruentus* crops grown across the Mbale dumpsite. Mean concentrations for Fe, Al, Zn, Mn and Cu were high in the two crops whereas Pb, Cr, Co, Cd, As, Hg, Se and Ni were in trace amounts. Significant variations for Al, Zn, Fe, Cr, and Co concentrations in crop parts existed across the dumpsite.Al, Co, and Fe concentrations in *Amaranthus cruentus* leaves were higher at the dump centre (16.3, 16, 17 mg/kg) than at the slope (8.2, 9, 7mg/kg) respectively. Zn concentrations in *Amaranthus cruentus* leaves were higher at the slope (120 mg/kg) than the dump centre (52mg/kg). Zn concentrations in *Zeamays* leaves were higher at the dump centre (21 mg/kg) than the river bank (5 mg/kg). Al and Fe concentrations in *Zeamays* seeds were higher at the dump centre (23, 14 mg/kg) than the river bank (10, 9 mg/kg) respectively. Al concentrations in *Zeamays* seeds were higher at the dump centre (23 mg/kg) than the slope (11 mg/kg) while Cr concentrations were higher at the slope (23 mg/kg) than the dump centre (14 mg/kg). Leafy crops accumulated most metals, with the overall occurrence higher at the dump centre. Pb concentrations were safe in *Zeamays* seeds, however, Cr, Pb, Zn and Al in the remaining crop parts were above World Health Organization/Food and Agricultural Organization (WHO/FAO) consumer food safety limits. *Zeamays* and *Amaranthus cruentus* crops grown across the Mbale Dumpsite could pose health risks to consumers. Routine assessments are required to ascertain potential health risks towards local food safety for human consumption. Key words Toxic metals, waste dumpsite, health risk, Uganda

Bees, Bugs and Beneficials in Environmental Risk Assessment and Testing (P)

TU291

Indirect effects of glyphosate-based herbicide on plant-herbivore interactions and oxidative status of the Colorado potato beetle

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Glyphosate is the globally most used herbicide against a wide range of weeds. Glyphosate has been considered safe to animals as it mainly targets physiological pathways in plants. However, recent toxicological studies have shown that glyphosate can cause various toxic effects also on animals. Glyphosate can also affect directly the synthesis of plant defence chemicals. In this study, we investigated the indirect effects of glyphosate-based herbicide (GBH) on non-target plant-herbivore interactions and the GBH-induced physiological stress on herbivores, by using potato and Colorado potato beetle (CPB) as a model system. The CPB larvae were randomly divided into two groups: **1)** environmentally relevant dose of GBH (Roundup® Gold, 450g/l) and **2)** control group (water). Larvae were exposed to GBH via potato, planted either in clean or GBH treated soil. Half of the larvae were reared on potato until the 4th instar and the other half was reared into adults to study the long-term effects of GBH on CPB. Plant defence chemicals were measured twice from the potato leaves; before placing larvae on potatoes and after larvae were pupated. GBH treatment had no effects on survival and body mass of the larvae or the adults. In larvae, glutathione concentration and the enzyme activities of catalase and superoxide dismutase were increased in GBH treated group, suggesting GBH-induced activation of antioxidant enzyme activities and elevation of glutathione (GSH) levels to defend the beetles against oxidative stress. However, glutathione-S-transferase, glutathione- peroxidase and reductase activities, GSH:GSSG ratio and lipid peroxidation levels were not affected by GBH treatment. In adults, none of the oxidative status parameters were associated with GBH. From the potato defence chemicals, α-solanine levels were reduced in potatoes grown in GBH treated soil, whereas α-chaconine levels were not affected by GBH. The reduction of α-solanine levels can be advantageous for the beetles, but detrimental to plant defence against herbivores. Potato defence chemicals differed also between the time points. Both α-solanine and α-chaconine levels were lower in later time point (both treatment groups), which may be related to larval feeding or potato growth. To conclude, environmentally relevant concentrations of GBH are not likely to affect directly the survival of CPB but are associated with the oxidative status of the larvae. However, GBH had no carry-over effects from larvae to adults.

TU292

Lethal and sublethal effects of two insecticides to predatory bug Anthocoris nemoralis in laboratory

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The management of most relevant insect pests of pear orchard in Emilia-Romagna

(Northern Italy) is achieved by insecticides. Therefore, the knowledge of the impact of pesticides on beneficial insects is a key issue in pear pest management. Predatory bugs (*Anthocoris* spp.) are key predators of the pear psyllid *Cacopsylla pyri* (L.) (Rhynchota: Psyllidae). In order to enhance conservation biological control it is important to assess the potential short- and long-term detrimental effects of insecticides used in pear orchard. This laboratory study focuses on possible effects of two insecticides on adults of *Anthocoris nemoralis* (F.) (Rhynchota: Anthocoridae), which is the most important predator of pear psyllid in Italy and potentially able to contain this pest under the economic threshold. Laboratory experiments were carried out to evaluate the lethal and sublethal effects of sulfoxaflor (Isoclast™ active, applied at 48 mg AI L^{-1}) and imidacloprid (Confidor 200 SL, applied at 100 mg AI L^{-1}) on *A. nemoralis* adults. The experimental unit consisted of a small pear plant (≈30 cm high) placed in an aerated plexiglass cylinder. Immediately after insecticide spray drying, 20 adult bugs were released into each cylinder and exposed to insecticide residues; 4 replicates were carried out for each product. Mortality was scored after a 72 h exposure. The survived bugs were removed and reared to study the impacts on life table parameters (i.e. fecundity and fertility). Key words: *Anthocoris nemoralis*, toxicity, sublethal effects, Isoclast

TU293

Lethal and sublethal effects of sulfoxaflor on the ladybird Adalia bipunctata

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The aphid predator *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) is a key natural enemy in agricultural and natural ecosystems and it is widely commercialized in Europe for augmentative biological control of aphids. Sulfoxaflor is a new active ingredient (IRAC 4C group) particularly effective against sucking insect pests. The assessment of its compatibility with aphid predators is crucial in developing integrated pest management programs on several crops. In this study, lethal and sublethal effects of sulfoxaflor on *A. bipunctata* were assessed under laboratory conditions; imidacloprid was included as reference product. Both the standardized method proposed by the IOBC and a demographic based approach, were used. The side effects of the insecticides were evaluated by directly spraying 3rd instar larvae and adults with the field rates recommended on product labels: imidacloprid (Confidor 200 SL: 100 mg AI L^{-1}) and sulfoxaflor (Isoclast™ active: 24 and 48 mg AI L^{-1}). Sulfoxaflor resulted harmless (IOBC class 1) for both *A. bipunctata* larvae and adults, whereas imidacloprid was classified as slightly harmful (IOBC class 2) for larvae and moderately harmful (IOBC class 3) for adults. None of the population parameters, R_0, r_m, λ, T, and DT, resulted affected by direct exposition of *A. bipunctata* adults to sulfoxaflor. On the contrary, imidacloprid caused a significant reduction of population growth indices mostly due to higher mortality and reduced adult fecundity in comparison to control group. Our results lead to the conclusion that, unlike imidacloprid, sulfoxaflor could be taken into consideration in IPM programs where *A. bipunctata* is involved. Key words: Integrated Pest Management (IPM), sulfoxaflor, imidacloprid, side effects.

TU294

Activity and persistence of two insecticides on Macrolophus pygmaeus and Aphidius colemani in laboratory and semifield experiments

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The selectivity of insecticides for beneficial arthropods is particularly relevant for glasshouse crops where the knowledge of the persistence of the toxic effects is necessary to synchronize and integrate beneficial releases together with insecticide sprays. In order to evaluate the selectivity of two insecticides, trials were carried out on *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae), an aphid parasitoid, and *Macrolophus pygmaeus* (Rambur) (Rhynchota: Miridae), a predator of whiteflies. Two insecticides were evaluated: Closer™ (sulfoxaflor Isoclast™ active: 120 g L^{-1}, DOW AgroSciences) and Confidor SL (imidacloprid: 200 g L^{-1}, Bayer CropSciences) at the concentration of 48 mg AI L^{-1} and 100 mg AI L^{-1}, respectively. Experiments to assess acute toxicity (expressed as percentages of mortality) were carried out on adults of both insects and nymphs of *M. pygmaeus*. The persistence of the harmful effects of insecticides was determined by exposure of nymphs and adults of *M. pygmaeus* and adults of *A. colemani* on treated tomato leaves at 1, 5, 15, 21 and 32 days after treatment (DAT). *M. pygmaeus* adults and nymphs were also exposed to tomato leaves grown after the treatments and thus not directly sprayed. These assays were carried out at 21 and 32 DATs. Five replicates with five individuals per species were performed for each treatment. Mortality was checked after 24 and 48 h of exposure for *A. colemani* adults and after 72 h for adults and nymphs of *M. pygmaeus*. On treated leaves, both insecticides fell in IOBC classes 3 or 4 for all DATs with a slight decrease of insecticidal activities overtime. Overall low toxicity was recorded for both insecticides on leaves grown after treatment. Both imidacloprid and sulfoxaflor were ranked in IOBC class 1 (harmless) for nymphs and in class 2 (slightly harmful) for adults of *M. pygmaeus*. Key words: *Aphidius colemani*, *Macrolophus pygmaeus*, sulfoxaflor, imidacloprid, side effects, greenhouse

TU295

A comparative study of the toxicity of the insecticide fipronil to aquatic Neotropical species

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Sugarcane cultivation has been highlighted in the Brazilian landscape with excessive application of insecticide fipronil. The aim of the present study was to evaluate the toxicity of Regent® 800 WG (a.i. fipronil) using a test battery of organisms that are native species with common occurrence in Brazilian freshwaters: Cladocera (*Ceriodaphnia silvestrii*), Oligochaeta (*Allonais inaequalis*), Diptera (*Chironomus sancticaroli*), Amphipoda (*Hyalella meinerti* and *Strandesia trispinosa*) and Amphibia (*Leptodactylus fuscus* and *Physalaemus nattereri*). All experiments were carried out under controlled temperature (25±1°C) and photoperiod (12h light:12h dark) and performed in triplicate. The test with *C. sancticaroli* was made with 240 mL test solution, 60 g artificial sediment and six organisms. For *H. meinerti*, 100 mL test solution was used with five organisms. Both tests lasted 96h. For *S. trispinosa* and *A. inaequalis*, the bioassays were carried in 10 mL test solution, with five organisms for 48h (*S. trispinosa*) and six organisms for 96h (*A. inaequalis*). For tadpoles, contained 5L test solution and five organisms for 96h. For cladocerans, consisted in five neonates for 96h. Species sensitivity distributions (SSD) were constructed to compare the acute EC50 values derived for the native species and other invertebrate and fish species. By means of the SSD, we observed that the species *Chironomus dilutes* is the most sensitive (0.03 µg/L), followed by *Americamysis bahia* (0.14 µg/L) and the *Cheumatopsyche brevilineata* (0.15 µg/L). With respect to native species, *H. meinerti* (1.29 µg/L) showed the greatest sensitivity, followed by *C. sancticaroli* (6.19 µg/L), *C. silvestrii* (9.7 µg/L) and *S. trispinosa* (53.24 µg/L). For *A. inaequalis, L. fuscus,* and *P. nattereri*, no effects were noted on the survival even at the highest concentration tested 100 mg /L for the oligochaetes and 800 mg/L for the tadpoles. Therefore, these species were the least sensitive to fipronil, being more resistant than the fish *Danio rerio* (181 µg/L) and *Oncorhynchus mykiss* (246 µg/L). Studies in Brazil recorded concentrations of 0.1 to 26.2 µg/L of fipronil in rivers in the state of Rio Grande do Sul and 4.23 µg/g in sediment of marginal lagoon of the river Mogi-SP. Based on these records, *H. meinerti* and *C. sancticaroli* would be at risk at such an exposure scenario. The present study demonstrated that toxic effects on tropical species are likely to occur at environmentally realistic exposure levels.

TU296

Severe effects of neonicotinoid insecticides on Nitocra spinipes under different exposure conditions

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Neonicotinoids have been constantly replacing formerly used insecticide groups (e.g. organophosphates, pyrethroids, etc.) in the past few decades. Their physical-chemical properties including lower log K_{OW} and higher water solubility make them readily absorbed by plants and act quickly at low doses. On the other hand, these properties make them susceptible for runoff and subsequent transfer into the aquatic environment. They have been detected in surface waters in the ng L^{-1} to low µg L^{-1} range, and finally in marine waters at concentrations ranging from 0.5 to 10 ng L^{-1}. In this work, we assessed potential acute and chronic effects of four neonicotinoids (clothianidin CLO, imidacloprid IMI, thiacloprid TCL and thiamethoxam TME) on the brackish copepod *Nitocra spinipes*. Neonicotinoid exposure was performed under three different scenarios. First in a 96h acute lethal toxicity test according to ISO 14669. Second in a recovery experiment based on 96h exposure to two different neonicotinoids (CLO and TCL) followed by transfer to fresh medium and continuous observation to explore the recovery potential of exposed copepods. Finally, we performed a 7-day larval development test with TCL according to ISO 18220. In the acute tests we found immobilization to be a more sensitive endpoint than death, showing EC_{50} (96h, immobilization) values ranging from 0.81 µg L^{-1} (CLO) to 430 µg L^{-1} (TME). Our recovery experiment showed that 69 % of the organisms exposed to 100 mg L^{-1} TCL were able to recover mobility after 24h, while those exposed to 100 mg L^{-1} of CLO remained immobilized. The larval development test with TCL showed a clear delay in development of *N. spinipes* nauplii and the EC_{50} (7d) was calculated at 10.3 µg L^{-1}, while the NOEC (7d) was 1.23 µg L^{-1}. *N. spinipes* showed considerable sensitivity when exposed to neonicotinoid insecticides both in short- and long-term exposure for adults and nauplii, respectively. Immobilization was a clearly more sensitive endpoint as compared to death in acute testing and locomotion is crucial for copepod survival in terms of feeding behaviour and predator avoidance. Regarding the recovery potential, there seems to be no general agreement for different neonicotinoids. Developmental effects of TCL on copepod nauplii suggested further testing of other neonicotinoids and particularly CLO (lowest acute EC_{50}) under chronic exposure conditions.

TU297

Working with EPT-Taxa - chronic testing of mayfly species in a test system simulating running waters

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As many other non-target arthropods, mayfly larvae originating from small creeks and streams are exposed to releases of plant protection products, which are mainly applied to agricultural areas and washed off into adjacent running waters. Even though mayfly species are considered to be very sensitive against exposure to several pesticides, there are only few toxicity data available dealing with chronic effects of pesticides on these species. Therefore, we developed a test system for the chronic testing of lotic insect larvae. The test system differs from usually known stream test systems particularly in its construction. In order to create a particular flow, small cages inside a test container filled with test medium circulate through the water phase thereby creating the target flow in the cage. The cages are equipped with gravel mats on the bottom and two black glass nuggets to simulate the natural habitat conditions of the larvae. Each cage serves as individual compartment for testing of single organisms to allow an organism specific evaluation of sublethal effects. The test system can provide data of chronic toxicity tests with different aquatic insect larvae, which can be used for a SSD (Species Sensitivity Distribution) approach for the risk assessment of pesticides. The chronic effects of the neonicotinoid Imidacloprid on field collected mayfly larvae of the species *Epeorus* and *Ecdyonurus* belonging to the family of Heptageniidae were assessed in a 28 day exposure period. Larvae, which were in an early development stage, were adapted to laboratory conditions for up to 14 days before test start. The study was carried out in moderately hard reconstituted water (according to EPA) with five test concentrations and a control. The grazing larvae were fed with green algae *Desmodesmus subspicatus*, which were grown on the glass nuggets before test start. As sublethal endpoint, the growth of the test organisms was evaluated by measuring the length of each individual larvae at test start and test end. In addition, mortality of larvae was recorded weekly. In previous studies, the test system was already used for chronic toxicity testing with stonefly larvae *Protonemura sp.* exposed to Imidacloprid for 21 days. In addition to the results of the performed mayfly study, a comparison of effects and sensitivities of mayfly and stonefly larvae to Imidacloprid will be presented.

TU298

Kinetics and toxicity of metabolites of imidacloprid to mayfly larvae

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Neonicotinoids (NNIs) belong to the group of nitroguanidine systemic insecticides, which recently sparked increased environmental concerns, based on their potential risks to the aquatic and terrestrial ecosystem. However, most attention has been given to the parent compounds and less is known of the impacts of their degradation products and metabolites. Since IMI is the most used NNI, we will study the toxicity of imidacloprid (IMI) and its metabolites. A mayfly species is chosen as the focal species, as it is amongst the most sensitive species for this compound and also an essential freshwater invertebrate. During upcoming experiments, the uptake kinetics of IMI and metabolites into the mayfly will be quantified and the toxicity of the parent and its metabolites will be assessed. Our results will provide a further mechanistic understanding of the toxicokinetics and toxicity of IMI and its metabolites in mayflies. To answer the question of whether IMI is degraded or metabolised by mayflies, and whether the mother compound or its metabolites is toxic, three types of experiments will be performed. Firstly, the toxicity of each chemical (IMI-olefin, IMI-urea, 6-chloronicotinic acid, 5-Hydroxy-IMI and IMI) will be assessed by a 4 days standard toxicity experiment. Secondly, the bioaccumulation of metabolites and environmental transformation products of IMI will be identified by analysing body burdens using Liquid Chromatography-Tandem Mass Spectrometry in a four days exposure study using the EC10 as the exposure concentration. Thirdly, those metabolites of IMI which accumulated in the mayflies or showed a high toxicity will be studied further in an uptake-elimination experiment to evaluate their toxicokinetics in order to get a better understanding and evaluation of the risks these chemicals may pose to aquatic organisms. The current study will (1) show the toxicity of the metabolites of IMI to mayflies, indicating whether metabolites are of importance for future risk assessment of IMI, (2) assess body burdens of IMI and its metabolites for mayflies, (3) show whether the degradation products of IMI in water are different from the metabolites found in the mayflies. This information might explain the high sensitivity of mayflies in comparison to, for instance, Daphnia.

TU299

Developmental and vitamin A-based biomarkers for pesticide contamination in two potential arthropod sentinels, the honey bee Apis mellifera and the gammarid Gammarus fossarum

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The massive use of pesticides is raising awareness for terrestrial and aquatic non target species. In response and to ensure efficient biomonitoring of ecosystems, the development and routine utilization of diagnostic tools (biomarkers) are essentials. Retinoid compounds (for example vitamin A) are implicated in many crucial physiological processes in vertebrates (development, reproduction, vision, etc.) and were proposed as sensitive biomarkers for several contaminants in fish, birds, amphibians and mammals. Recent findings regarding fluctuating levels of retinoids in bees following pesticide exposure open avenues of research for new biomarkers in invertebrate sentinel species. In this project, we propose to study the retinoid system of two arthropods; an insect (*Apis mellifera*) and a crustacean (*Gammarus fossarum*). In the first place, we will establish the retinoid profile of these models throughout their development. Secondly, the implication of retinoids in the embryonic, morphogenetic and sexual development following exposure to retinoic acid (bioactive form of vitamin A) or citral (retinoic acid synthesis inhibitor) will be investigated. Lastly, we will assess the sensitivity of the retinoid system to glyphosate and methoprene. These results will lead to the proposal of new biomarkers to complement biomonitoring programs.

TU300

Analysis of ecotoxicological studies with non-target arthropods based on the PIERIS databank

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The ecotoxicological risk assessment of plant protection products (PPP) for non-target arthropods (NTA) in Europe is basically based on ESCORT 2 (2002). Since the publication of ESCORT 2 many adjustments have been implemented and new knowledge and concepts published (e.g. ESCORT 3 (2012) and EFSA Scientific Opinion (2015)). However, new approaches have not yet been incorporated into the risk assessment within the regulatory context. Before doing so, it is important to validate current and suggested new approaches for their usefulness, feasibility and consequences for the authorisation of PPP. PIERIS (Pesticides and Intermediates Environmental Risk Indication System) is a databank containing ecotoxicological information about the PPP and active substances evaluated as part of the authorisation process in Switzerland. PIERIS has been constantly developed and expanded since 2002. Today PIERIS contains information about the physio-chemical properties, environmental fate and application parameters of over 400 active substances, 800 metabolites and 1200 PPP. Furthermore, in total over 24000 ecotoxicological studies, of which ~4400 are related to NTA, have been entered in the databank making it an excellent tool for testing different questions. The aim of this work is to use data from PIERIS to investigate following aspects related to the risk assessment for the NTA: 1) Interspecific variation in sensitivity towards different active substances. 2) Comparison between different mode of action types. 3) Comparison of conclusions on recovery based on different study types (glass plate, extended, aged residue, field studies) 4) Influence of different VDF-values (vegetation distribution factor) on the outcome of the risk assessment The results will enable us to better interpret risk assessments according to Escort 2 und to judge the pros and cons of new approaches.

TU301

Sampling methods for Collembola: Comparing abundance, diversity and variability

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The functional role of Collembola in agro-ecosystems is diverse and important. Collembola function in plant litter decomposition processes, in forming soil microstructure, and are key in arthropod food-webs. Species differ in habitat use. Whereas some are predominantly surface dwelling, others are mainly hypogean and again others may commute between compartments. Different sampling techniques exist, and each has their own bias. For example, pitfall traps will target surface dwellers and hypogean traps or soil cores will target euedaphic species. For regulatory studies, with spray applied products both surface and soil dwelling species will be at risk and it is not a priori clear which of the available methods will be best suited. The suitability of a sampling method is determined by the effectiveness of catching the Collembola taxa of concern. Abundance and diversity of taxa sampled, and variability of the data determine the quality of the risk assessment: the array of taxa occurring in the field that are actually covered by the risk assessment, and the detection level of potential adverse effects. In this work we compare results of three sampling methods for collecting Collembola from the soil surface (soil cores, pitfall traps and suction samples). Data are from two arable studies (winter wheat) performed in two distinct geographical locations (South-West France and The Netherlands). Quality of the data generated with the three sampling methods are evaluated, in terms of total numbers, array of taxa covered with the sampling methods, number of taxa having sufficient numbers for meaningful effect evaluations, and variability of the data. Abundance and heterogeneity of the data greatly determine detectability of treatment effects. Variability of the data is compared by calculating minimum significant differences (MSD); the minimum effect value that would be detectable in a statistically significant manner, given the variability of the data. Based on MSD values, data quality of taxa from the different sampling methods is then classified according to Brock *et al*. (2015) and Bakker and Aldershof (2018). Finally, effect values calculated for identical taxa collected with the three sampling methods are compared, to investigate the level of exposure of taxa sampled with the different methods. Recommendations are given to improve data quality by using appropriate sampling methods for Collembola, in line with EFSA (2015).

TU302

What about Lepidoptera? Future requirements and proceedings on risk assessment towards Non Target Arthropods

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Ever since the European Food Safety Authority (EFSA) firstly published their “Scientific Opinion adressing the state of science of risk assessment on plant protection products for non-target-arthropods” in 2015, a number of challenges and yet to be researched improvements concerning the risk assessment and study designing have been raised for discussion. One aspect among numerous newly raised concerns comes with including a new group of organisms to risk assessment, the large and important group of *Lepidoptera* species. The EFSA working group “considers *Lepidoptera* to be important drivers for the ecosystem services pollination and food web support”, but yet validated and reliable testing protocols for oral and contact exposure are missing, not even speaking of harmonized and accomplished official ringtests on broad international scale. A few methodologies for Lepidoptera testing exist and are also mentioned by EFSA (Appendix G), but these are either limited to special product use (e.g. *Bacillus thuringensis* strains), or not yet developed to high level end use (e.g. *Pieris brassica*). In Appendix G, the mentioned setup for *Pieris brassica* seems to be a promising approach, although it may be limited to oral exposure. Further on *Pieris brassica* is classified as pest organism to some farming cultures (e.g. cabbage) an aspect which counteracts to the overall combined approach of plant protection products and use of “beneficials”. Lastly, there are a lot of species from the *Lepidopteran* group (e.g. diamondback moths, cabbage loopers, tip moths) required for efficacy testing throughout registration process of plant protection products. The here presented work shall emphasize a possible approach for a test method on *Lepidoptera* species under oral exposure, based on an accepted and reliable chinese guideline method for silkmoths, *Bombyx mori* (ICAMA 2014). Further on, aspects and contradictions of requiring such a setup under risk assessment and registration for plant protection products for this specific group of organisms will be discussed.

TU303

Designing and conducting mesofauna field studies - what should study design consider in terms of sample storage, extraction and detectable differences?

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In cases where risks of plant protection products have been identified on the basis of laboratory tests with standard soil organisms, mesofauna field studies can be conducted as a higher-tier refinement option. In such studies, effects on the species composition and abundance of the concerned taxa are investigated at community/population level under realistic exposure conditions. While planning mesofauna field studies, different conceptual and technical aspects need to be considered. For the evaluation of such studies, statistical tests are conducted to check for the effects of the toxicants. However, the ability of a test to indicate a difference between treatment groups always depends on significance criteria, the number of replicates, and variance in the data. A suitable study should allow the detection of a relevant minimum difference (MDD) between treatment groups. Hence, it is crucial to apply a suitable sampling design (e.g. in terms of number of replicates or consideration of different soil horizons) to ensure an appropriate explanatory power of the study. Additionally, from the technical point of view, it is of interest if the orientation of soil cores during the extraction of organisms (i.e. either normal or upside down) or their cold-storage (compared to direct extracted samples) has an effect on the extraction efficacy. To investigate these aspects of the study design, soil core samples were taken from fifteen spots on a grassland. Four soil cores were taken from each spot and divided into an upper (0-5 cm) and a lower (5-10 cm) horizon. The four soil cores represented four different sample handling and storage conditions: (i) normal orientation of the sample during extraction (same as in the field), no storage, (ii) reverse orientation (upside down), no storage, (iii) normal orientation, storage under cooled conditions before extraction, and (iv) reverse orientation, cold-storage. After extracting Collembola and Acari species from the soil cores, the data was analysed to investigate if any difference between the different handling and storage conditions could be detected. In a second step, this data set, comprising a large number of replicates, was used to simulate different realistic sampling designs and illustrate the relationship between the number of replicates and the explanatory power (measured by the MDD) for this type of field study. Finally, the results are discussed in the light of mesofauna field study recommendations.

TU304

A semi-field bio-assay to evaluate effect decline of chlorpyrifos using honeybees exposed to aged residues on different flowering crops.

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Because wild bees and honey bees play an important role in the pollination of a wide range of crops and uncultivated plants there is concern over decrease in bee populations. To evaluate the risk of plant protection products *Phacelia* is recommended in Europe as a standard crop for bee studies. However, because flower morphology and longevity may determine exposure of honeybees to pesticide residues, bee-attractive crops with a different flower morphology should also be evaluated. In this experiment we used a test design that enabled a simultaneous assessment of the effects of different residue age classes on honeybee survival. The study design was a semi-field study with up to 6 treatment groups with 3 replicates each: chlorpyrifos treated at 750 g a.i./ha, 21, 14, 7, 3 and 0 days before the start of exposure to honeybees and an untreated control. Each replicate consisted of one gauze cage of 20 m2 with treated crop. Each exposure cage had an extension with 20 m2 of untreated crop separated by a gauze curtain, which was used to acclimatize the honeybees before the start of the exposure. A small bee hive was placed against the gauze separation. At DAT0 the curtain was simply moved over the hive, thus confining the bees to the previously treated crop. Application on DAT0 was done before bee flight. *Phacelia*, buckwheat, mustard and sunflower were selected based on different flower morphology and longevity. Assessments of foraging and mortality were conducted up to 5 days after the start of the exposure. For data analysis the total number of dead bees recorded 1day after exposure, corrected for control mortality was used. Because effects of different residue age classes could be assessed simultaneously, i.e. under exactly the same meteorological conditions, these effects could be compared in a straightforward manner and effect decline could be modelled using regression analysis. Mortality decline curves over time showed differences between the test crops which confirms that exposure of bees to residuals depend on flower morphology. The highest initial mortality was observed on buckwheat, which also showed the steepest decay curve, followed by *Phacelia*, mustard and sunflower. Mortality-based DT50 values were determined by fitting the data to kinetic degradation models, being 2.61 days for buckwheat and 4.09 days for *Phacelia*. The studies with sunflower and mustard had insufficient time points for the regression due to external circumstances.

TU305

Bee colony assessments with the Liebefeld method: How do individual beekeepers influence results and are photo assessments a possibility to reduce variability?

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Colony strength, food storage and brood development are a fundamental part of each honeybee field study. Colony assessments are used to compare and assess those for beehive over time. At present, most colony assessments are made by experienced beekeepers according to Liebefeld. This method is based on an estimation of areas covered by honeybees, food and brood stages on each side of a comb. Areas are counted from a grid separating the comb side into 8 sections which are protocolled with an accuracy of 0.5 sections. An assessment for a hive takes up to 20 min and even with two field locations, it is necessary to split assessments between beekeepers. So, it is important to make estimates as comparable as possible. For this purpose beekeepers practice the assessments on pre-determined photographs to “calibrate themselves”. The advantage of the Liebefeld assessment is that the condition of bee hive is estimated with minimum disturbance of the bees. Digital photography is under discussion to gain data with high precision and accuracy with one major disadvantage. To be able to see food and brood stages in photographs, bees have to be removed from combs. This, however, results in a disturbance of the colony – especially if the assessments take place in short time intervals of 7 ± 1. An experiment was performed to evaluate the variation between individual beekeepers and to compare the results to data generated with photographs. For the experiment, five colonies were assessed each by five beekeepers independently according to Liebefeld method. Each comb side of the five colonies was photographed with and without honeybees sitting on it for precise analysis at the computer for a number of bees, nectar cells, pollen cells, eggs, open brood and capped brood. The number of bees and cells with the different contents were generated by an area-based assessment in ImageJ as well as a detailed counting with help of HiveAnalyzer® Software. Data from beekeeper estimations were then compared with assessments based on digital photography. With the results of the experiment, we tried to answer several questions. First of all, we wanted to determine the level of variation between the beekeepers for the live stages and food stores estimated. Furthermore, we wanted to find out, if the photo assessment is such a precise method that it would justify a replacement of the well-established Liebefeld method despite the strong disturbance of the bees.

TU306

Pollinator monitoring for evaluation of potential exposure and assessment of effects

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Over the last decades there has been a decline in arthropod numbers observed. Pollinators are one of the groups that were clearly affected. At the moment the safety of plant protection products is evaluated with the testing of honeybees but other pollinator species are not required. There are already some test designs in development that use bumble bees and mason bees but they are not suitable for all crops and seasons. Pollinator monitoring can be a way to evaluate potential exposure of pollinators to plant protection products in different crops or agricultural scenarios or to assess possible effects of treatments at a larger scale taking into account other influencing parameters like the management intensity, landscape structure etc. In a monitoring, the abundance and species richness of natural occurring pollinators in a crop and adjacent field margins will be investigated. Crops can be used by pollinators not only for foraging but also as a nesting habitat. Additionally, even the off-crop habitat close to a field can be attractive for the same reason. Therefore the assessment of pollinator abundances in both field and the surroundings can help to understand if pollinators are exposed to plant protection products. This might include temporal as well as spatial differences (timing of monitoring and placement of monitoring within the field and landscape). To evaluate a wide range of pollinator species occurring in a specific crop several methods are available and needed. As with non-target arthropod studies it is recommend using different types of assessment methods to collect the most representative community. Sampling should include non-selective and selective methods. For the non-selective methods different types of traps might be used in combination: vane traps and bee bowls. These traps can be installed at different locations: i.e. in the center, at the borders of the fields and outside in the adjacent field margins. As selective method sweep netting or observations can be used via transect walks in a defined distance and time interval. In addition, trap nests can be provided for hypergeic solitary wild bee species that breed in woody cavities. The trap nests can also be set up at the different locations and are used for sampling of pollen to assess pollen sources by pollen identification of pollen mass samples. If required, residue analysis can performed with samples of pollen mass. This would allow assessing possible exposure though a test item.

TU307

Honeybee's biological and behavioral processes can affect the outcome of the RFID homing flight test

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The development of new ecotoxicological test-methods with honeybees requires the inclusion of fundamental bee biology and corresponding behavioral processes, in order to improve the explanatory power of a new test-method as much as possible. Generally, in ecotoxicological (lower-tier) studies with honeybees, xenobiotic substances are administered via group feeding to a certain number of bees, which distribute the test solution between each other via trophallaxis. Accordingly, group feeding represents one of the main principles in the RFID homing flight ring-test protocol "Homing flight test in honeybee (*Apis mellifera L.*) after single exposure to sublethal doses (J. Fourrier, 2018)", whereby the test-substance is administered in sugar solutions to groups of ten foragers. Due to methodological reasons of the present protocol, the age- and task composition of collected foragers may not be homogenous. As a result, corresponding food uptake could vary considerably among individual bees and/ or groups. Similarly, group-based food distribution via trophallaxis might not distribute the feeding solution homogenously among individual bees, as it has been pointed out in previous studies. The performance of slightly modified experiments based on the RFID homing flight ring-test protocol represents a good way to evaluate the effect of an individual parameter on the outcome of this new test-method. \n We demonstrated that the pooled homing ability of xenobiotic exposed foragers differed significantly when administered individually compared to group feeding. Furthermore, we showed that the current content in nectar stomachs of returning nectar foragers (collected for the trials) can affect the data (e.g. due to dilution of the test substance, or the likeliness of individual bees to take up the feeding solution). Our results bring new insights, how the RFID homing flight ring-test protocol could be improved, using individual feeding and /or choosing exclusively returning pollen foragers to create equivalent test units.

TU308

Oral Toxicity Test with Solitary Bees: Long-term feeding behavior

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As the current version of the ICP-PR working document on the Oral Acute Toxicity Test with Solitary Bees is being prepared to become an OECD guideline, there are still practical issues with the test, most notably that all participants of the ring test experienced a high number of bees which do not feed on the provided solution in the given time. Two small-scale experiments over 72 hours were performed. In the first, different concentrations of sugar solutions were tested. In the second, it was checked if consumption becomes more robust over longer time periods. Looking at longer time periods had the intention of considering if a chronic test with solitary bees would be possible, but also assessed if prolonging exposure or doing a pre-exposure phase in the acute test would lead to a better consumption. Overall, the tests showed that once bees start to feed they will continue to feed over the following days. This is an important first step for the possible establishment of a chronic test. For the acute test, it shows that introducing a pre-exposure phase in which the bees can "learn" to feed would increase the number of feeders and reduce the overall number of bees required for one test. There were, however, still bees that starved to death instead of feeding on the provided sugar solution (or offered alternatives), so there is still a need to investigate how consumption can be increased.

TU309

Determination of abamectin and difenoconazole in tissues of the stingless bee Melipona scutellaris

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Studies involving the assessment of adverse effects of agrochemicals in native bees are scarce in Brazil. Thus, because of the important role in pollination process, investigation about the bee's susceptibility to agrochemicals commonly applied in crops are important. In this study an analytical method was developed for the determination of the insecticide abamectin and the herbicide difenoconazole in tissues of the stingless bee *Melipona scutellaris*. For this purpose, QuEChERS acetate modified method was used for sample preparation and the pesticides were determined by LC-MS/MS. The validation parameters of tested method include: a linear range between 0.01 to 1.00 mg L^{-1}, with correlation factors above 0.99; detection and quantification limits for abamectin and difenoconazole of 0.003 and 0.01 mg L^{-1} respectively; recovery of 55.5 % for abamectin and 106.7% for difenoconazole, with an intraday accuracy ranging between 1.44 to 10.50% for abamectin and 3.19 to 7.55% for difenoconazole. Considering these parameters, this validated method can allow de detection of concentrations below the regulatory agencies stablished levels (0.02 mg L^{-1} for abamectin and 0.5 mg L^{-1} for difenoconazole) for crops as strawberry fields, where bee pollination is extremely important. Thus, method was applied in bees exposed via oral (0.006 $\mu g\, \mu L^{-1}$ and 5.0 $\mu g\, \mu L^{-1}$) and topic (0.028 $\mu g\, \mu L^{-1}$ and 67.0 $\mu g\, \mu L^{-1}$) at LD_{50} concentration for abamectin and difenoconazole respectively. Analytes were detected in all tested conditions indicating the method can be applied for the monitoring of these two pesticides in bee tissues.

TU310

Validation of analytical procedure for the determination of Abamectin and Difenoconazole in strawberry flowers (Fragaria ananassa) and pollen sampled from Scaptotrigona depilis hives

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The increase of agricultural productivity associated with the emergence and the extensive use of agrochemicals is undeniable. In this sense, the harmful effects, as well as the impacts of those applied toxic agents over the ecosystem, was laid aside in the first moment. However, strong evidence indicates that the rising use of agrochemicals is one of the factors associated with the decreasing of pollinating insects, such as bees, which is considering as the animal that contributes most significantly to this process and is increasingly scarce. The present work aims the development and validation of an analytical procedure for the determination of the insecticide abamectin and the fungicide difenoconazole in strawberry flowers (*Fragaria* x *ananassa* DUCH.) and pollen sampled from beehives of the stingless bee *Scaptotrigona depilis*. The QuEChERS method was optimized for extraction and clean-up procedures, and analytical performance and determination of the two agrochemicals were verified using LC-MS/MS. For the method validation, obtained results involving linearity (0.5 to 1500 $\mu g L^{-1}$), precision (>80%), accuracy (< 20%), and recovery in three spiking levels (250, 1000 and 1500 $\mu g L^{-1}$) were satisfactory and in accordance with international guidelines on validation. Matrix effect (>74%) was observed for both contaminants in the two matrices. Thus, the method can be applied in further studies, being an important tool for the determination of these agrochemicals on investigated matrices and contributing to the environmental risk assessment involving these organisms.

TU311

Evaluation of honeybee larvae data: sensitivity to PPPs and impact analysis of EFSA Bee GD

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Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the risk on honeybee larvae or honeybee brood. In July 2013 the European Food Safety Authority (EFSA) published a guidance document on the risk assessment of plant protection products on bees (EFSA 2013). This document is intended to provide guidance for notifiers and authorities in the context of the review of plant protection products (PPPs) and their active substances under Regulation (EC) 1107/2009 (EC 2009). The first objective of this poster is to summarize all available industry data for active substances and formulated products on honeybee larvae testing according to OECD 237 and OECD 239, in order to gain an overview of these results and the selectivity of different product groups. The sensitivity of the endpoints are presented. In addition, endpoints obtained at different development stages after 7 and 22 days in OECD 239 studies are compared. As a first step in the risk assessment, EFSA requires a screening step, which consists of the calculation of risk quotients (ETRs) for honeybee larvae. This considers exposure routes for the in-field (PPPs applied as sprays) and off-field (PPPs used as seed treatments and granules) scenarios. Where a use does not pass one of the screening level risk quotients, EFSA offers the possibility for refinement in a tier I risk assessment. This includes the refinement of exposure estimates from the screening step and also additional exposure routes, such as the exposure to flowering plants in the field margin and adjacent crops. As worst-case scenarios, the risk of honeybee larvae being exposed to treated crops and weeds were assessed. The same approach as used for the honeybee was also conducted for bumble bees and solitary bees but with the application of specific short cut values (SV) from the EFSA guidance document. As honeybee endpoints are used as a surrogate for bumble bee and solitary bee data an additional 10x safety factor is applied to the endpoints. The second objective of this poster is to evaluate the impact of the proposed screening and tier I risk assessments on the pass rates of currently available active substances and formulated products, which is an ability of the scheme to correctly identify compounds of potential concerns and consequently screen out those of low concern. In addition, the outcome of an industry proposed alternative risk assessment as described by ECPA (2017) is presented.

TU312

Chronic oral exposure of adult honeybees to PPPs: Sensitivity and impact analysis based on EFSA Bee GD

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Based on EU Regulation 1107/2009/EC the current regulatory risk assessment on bees has to address the chronic risk on adult honeybees. In July 2013 the European Food Safety Authority (EFSA) published a guidance document on the risk assessment of plant protection products on bees (EFSA 2013). This document is intended to provide guidance for notifiers and authorities in the context of the review of plant protection products (PPPs) and their active substances under Regulation (EC) 1107/2009 (EC 2009). The first aim of this poster is to summarize industry data based on studies conducted up to 2018, for active substances and formulated products on the chronic oral testing of adult honeybees according to OECD test guideline 245 and its previous drafts, in order to gain an overview of these results and the selectivity of different product groups. As a first step in the risk assessment, EFSA requires a screening step which consists of the calculation of risk quotients (ETRs) for the chronic exposure based on the application rate, an application depending shortcut value, an exposure factor and the endpoint (LDD_{50}). This considers exposure routes for the in-field (PPPs applied as sprays) and off-field (PPPs used as seed treatments and granules) scenarios. Where a use does not pass one of the screening level risk quotients, EFSA offers the possibility for refinement in a tier I risk assessment. This includes refinement of the exposure estimates from the screening step and also additional exposure routes, such as the exposure to flowering weeds in the field and adjacent flowering crops. Screening step and tier I risk assessment were also conducted for bumble bees and solitary bees, using 1/10 of the honeybee endpoint. The second aim of this poster is to evaluate the impact of the proposed screening and tier I risk assessments on the pass rate of currently available active substances and formulated products, thereby testing the ability of the scheme to correctly identify compounds of potential concern and consequently screen out those of low concern. The third objective of this work is to present the outcome of alternative calculations as described by ECPA (2017). The aforementioned analysis follows the principles described in the ECPA impact analysis (Alix et al. 2013) which used theoretical data due to lack of real data. The present analysis compares the pass rates from this first approach with the outcome based on real laboratory data which are now available

TU313

Conservatism in first tier trigger values in pollinator risk assessment

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The EFSA guidance on the risk assessment of plant protection products on bees (EFSA,2013) provides examples on the calculation of trigger values used in risk assessment. These values are based on several assumptions, i.e., the normal background mortality, the amount of mortality that a hive can withstand without negative effects (7% derived from the Khoury model) and the effect the compound has on bees. The latter is the focus of our current project. Where effect curves are standard of a sigmoid shape and are being normally analysed using non-linear regression techniques, the EFSA guidance adopts another approach and assumes a linear dose-response curve. As already stated in the guidance, this introduces a certain level of conservatism in the risk assessment because an effect level derived from a linear approach will result in a lower concentration than when this level is derived from the standard non-linear approach. In order to test the amount of conservatism introduced this way, we analysed data from sixty-two 10d-chronic honeybee tests using both the linear and the non-linear approach. From this data set fifty-two effect curves were obtained comprising 18 fungicides, 13 herbicides and 21 insecticides, which were processed following the examples provided by EFSA. Comparing the results of the linear and non-linear approach showed that using the linear approach gave more conservative results, ranging from a factor 0.7 to 33.4 for individual compounds. On a whole the ETR trigger was a factor of 13.3 more conservative using the linear approach proposed by EFSA as when the normal linear approach was used.

TU314

Optimizing laboratory testing for bee species: a comparative sensitivity analysis for honey bees and bumblebees

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A data evaluation was conducted by European Crop Protection Association (ECPA) member companies to compare the sensitivity of bumblebees (*Bombus terrestris* L.) with that of honey bees (*Apis mellifera* L.) to pesticide active substances and plant protection products. For the evaluation endpoints from acute oral and contact laboratory toxicity tests with honey and bumblebees were compiled into a database. Studies were included where there was at least one corresponding test for both species according to the same route of exposure. This resulted in 108 test items for contact exposure and 97 for oral exposure. The data comprised of approximately 20% fungicides, 50% herbicides/plant growth regulators, and 30% insecticides/nematicides). For data sets with definite endpoints (i.e. calculated LD_{50} values) the sensitivity ratio "SR" was determined by dividing the honey bee LD_{50} value by that of the bumblebee LD_{50} value. The data evaluation indicated that bumblebees were of similar or lower sensitivity compared to honey bees for all fungicides, herbicides/plant growth regulators and for 86% of insecticides/nematicides irrespective of the route of exposure. Overall, most of the comparisons indicated that bumblebees were not more sensitive than honey bees based on an acute toxicity assessment. We conclude that based on this review routine regulatory testing of bumblebees (*Bombus terrestris* L.) of plant protection products is not justified and the application of a standard safety of 10 on honey bee endpoints to cover bumblebee sensitivity is unwarranted.

TU315

Applying the mechanistic honey bee colony model BEEHAVE to assess multiple factors impacting overwintering survival in large colony feeding studies

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Honey bee Large Colony Feeding Study (LCFS) is a novel type of Tier II semi-field study for the determination of potential effects of pesticides on free-foraging whole colonies during and after dietary intake of a known pesticide concentration. LCFS are currently accepted by North American regulatory agencies and represent a progressively more realistic level of refinement compared to individual laboratory-based studies. LCFS are designed to test toxicity via consumption of fed sucrose syrup over a worst case exposure scenario of six weeks, with colony assessments conditions over a foraging season and following overwintering period. However, such studies are very cost- and time-intensive, and high overwintering losses of control hives have been observed in some studies. Loss of control colonies indicates that stressors other than pesticides, e.g. resource availability, weather, diseases and beekeeping activities, likely influence colony overwintering survival, confounding the assessment of impacts caused by pesticides. In the current study commissioned by the Pollinator Research Task Force, we apply the mechanistic honey bee colony model BEEHAVE to simulate colony dynamics observed in negative control colonies from multiple colony feeding studies. Detailed landscape-level data inform the resource availability for the simulated foragers in the model. In addition, weather data, initial colony condition and feeding patterns were analyzed across studies and translated to model inputs. In a calibration step, we adjusted parameters in BEEHAVE to achieve simulated dynamics corresponding to colony conditions reported in the studies. Study data collected in summer and fall were analyzed for predictors of overwintering success of individual colonies. BEEHAVE simulations with different combinations of external factors were used to assess their importance for colony condition. Colony conditions at study initialization and feeding patterns both influenced the colony condition in the fall, and thus, the probability of overwintering survival. Model simulations with different colony feeding patterns and initial colony conditions were then used to quantitatively estimate colony-level outcomes under conditions deviating from those in the studies. These results can be used to improve and inform LCFS study designs. Pesticide effects can be included in future model analyses, and analyzed in the context of multiple factors that impact colony health and overwintering success.

TU316
Challenges in the modelling of multiple stressors on the honeybee colony
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The Western Honeybee (*Apis mellifera*) is a key species of interest in the risk assessment of pesticides in part due to its major role in pollination services, a vital part of agriculture worldwide. The honeybee colony is a complex societal structure, consisting of tens of thousands of individuals each performing specific tasks depending on their age and the current state of the colony (food stores, food availability, time of year). The success of the colony stems from the complex interactions between these individuals and the ability of the entire colony to collect sufficient food to feed the brood and survive over the winter, as well as to maintain the homeostasis of the colony (cleaning, tending of brood, temperature control). In a typical year, each colony is subject to multiple stresses, from food availability to parasitism and predation, as well as exposure to pesticides. Due to the highly interwoven and complex nature of the colony, it is clear that the use of ecological models to understand the relative impacts of various stressors on both the individual and colony level is highly beneficial. The BEEHAVE model was developed to represent honeybee colony dynamics by integrating relevant colony processes into one framework. The model has a simple interface allowing non-expert users to explore colony dynamics and quantify impacts of various stressors. Since its release, the BEEHAVE model has been used to explore a number of stressors, as well as undergoing a review by EFSA, to explore its potential as a tool for pesticide risk assessment. The design of BEEHAVE permits the inclusion of multiple stressors acting simultaneously on the colony, and can capture simple acute impacts well. However, when quantifying more complex, chronic stresses, there are a number of challenges that need to be addressed. In modelling the honeybee colony, there are a number of parameters that are required to correctly capture the state of the colony at any specific point in time. Specifically, for an Individual-Based Model, such as BEEHAVE, the age structure and available resources within the colony, as well as the parameterisation of the landscape and weather are significant drivers of colony dynamics and often are difficult to derive from empirical data. In this poster, we discuss these challenges and possible ways of capturing the inner workings of the colony in a sufficient resolution to adequately scale impacts from individual bees to long-term colony dynamics.

TU317
CORAL: innovative open source QSAR model for predicting acute contact toxicity of binary mixtures of plant protection products in honeybee (A. mellifera)
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Honeybees can be exposed to a wide range of multiple chemicals "chemical mixtures" as compounds from anthropogenic origin (e.g. plant protection products, PPPs) or natural origin (e.g. mycotoxins). Quantifying the impact of multiple chemicals on bees has been identified by EFSA as a priority to understand their relative contribution to bee health in comparison to other stressors (e.g. varroa) to support the development of holistic risk assessment method. However, from a risk assessment perspective, it is practically impossible to test all the possible mixtures experimentally, therefore it is needed to find smart strategies to assess the potential hazards using new strategies that incorporate alternative methods such as *in silico* tools. Here we propose the application of CORAL QSAR model in order to predict the combined effects of PPPs in honeybee (*A. mellifera*). Acute contact toxicity data for 92 PPPs binary mixtures ($LD_{50\text{-mix}}$) and corresponding single chemicals ($LD_{50\text{-single}}$) were retrieved from the literature and used to build the QSAR model. Similarly, optimal descriptor as well as quasi-SMILES were estimated. Three random splits into the training, invisible training, calibration, and validation sets were generated in order to test the statistical quality of the QSAR model. Preliminary results showed a R^2= 0.76 for the validation test. Finally, Toxic Unit (TU) approach assuming Concentration Addition (CA) as default model was applied in order to identify deviations of effects from dose addition that lead to synergistic or antagonistic interactions. This work shows the potential use of alternative methods to animal testing such as QSAR models for the hazard assessment of multiple chemicals. In particular, when applying a tiered approach for ecological risk assessment of multiple chemicals, QSARs models can be applied in order to a) predict (missing) information on individual compounds (tier 0) and (b) predict directly or stepwise the combined effects and interactions of chemicals in the mixture (tier 1). Overall, this project will allow EFSA's Working Group "MixTox" to develop its Guidance Document aimed at harmonizing the current methodologies for HHRA and ERA of multiple chemicals due in 2019.

TU318
Modelling of exposure to pesticide residues using citizen science based monitoring of pollen - part of the INSIGNIA project
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In the INSIGNIA EU project, a protocol will be developed and tested for citizen-science based monitoring of pesticides using honeybees. As part of the project, biweekly pollen samples will be obtained from sentinel apiaries over a range of European countries and landscapes, validating different devices and methods. The samples will be analysed for botanical origin, using state of the art molecular techniques, and for residues of a large number of agricultural pesticides and veterinary products, both authorized and unauthorized. The data collected will be used to develop and test a spatial modelling system aimed at predicting spatially-explicit pollen diversity and pesticide mixture exposure and risk at the apiary level, from basic landscape data. The system consists of model components for spatial and temporal pollen resource distribution, application and environmental fate of pesticides, and honeybee landscape-scale pollen foraging, with a common underlying geo-data base containing European land-use and land-cover data (CORINE) supplemented with national data sets on agricultural and (semi-)natural habitats. It requires (regional, national) data on the use of agrochemicals, as well as data on the phenology and other characteristics of the crops and wild flowers constituting the main pollen resources. The modelling system is designed to develop and improve incrementally, taking advantage of the growing amount of data becoming available from the sampling in the course of the INSIGNIA project (and possible future continuation), and iteratively, when a next round of comparison between observations and predictions from a set of alternative models indicates that the initially simple models, e.g., based on distance relations only, can be replaced by more complex and detailed ones, accounting for increasing ecological realism. The approach can generate risk maps based on floral resources and residues indicative of suitability of the landscape for pollinators. Comparison between model predictions and observations will indicate to what extent apiary exposure can realistically be predicted from relatively coarse-grained Europe-wide landscape data using models of intermediate complexity, and point out steps required to arrive at more precise predictions. Comparing results for authorised and unauthorised pesticides will provide insights into actual use of the latter, including the spatial distribution and the most likely crops on which they have been applied.

TU319
Understanding hive dynamics
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For the evaluation of the toxicity of chemicals honeybee field and semi field trials are routinely conducted in order to measure endpoints such as brood termination or colony strength. These rather simple endpoints are, however, a result of the incredibly complex interaction of a multitude of factors, ranging from climate, beekeeper practice, landscape structure and toxicants. As a consequence it is difficult to understand which of these factors have the most relevant impact on colonies, although in particular in the context of the debate on multiple stressors such insight would be valuable. In the present study we use computer generated visual representations of hives to illustrate how hives change over time to understand the relevance in particular of climate and hive structure. We also show how brood success is defined by hive structure.

TU320
Bee keepers and other stressors to honeybees
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In recent years a number of large scale national and international projects (e.g. COLOSS) identified the bee keeper training or practice \n as the most relevant factor regarding colony losses. To elucidate the relevance of factors such as bee keeper practice, Varroa treatment, \n weather and toxic stressors a spatially explicit population model is used to compare the relevance of these factors for the colony survival.\n Simulations with different weather scenarios, varying bee keeper practice and exposure to toxicants are conducted. Results of model \n simulations are discussed.

Climate Impacts on Polar and Alpine Ecotoxicology and Environmental Chemistry (P)

TU321

Biomagnification of PFAS in the Antarctic breeding south polar skua

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Exposure to manmade chemicals in the Antarctic ecosystem has historically been low, but the Antarctic breeding south polar skua (*Catharacta maccormicki*) has concentrations of some chlorinated biomagnifying lipid soluble contaminants comparable to Arctic breeding seabirds at similar ecological niches. There are few data on the protein soluble per-fluorinated compounds PFAS from Antarctic wildlife, however a previous study of south polar skua found increasing PFAS concentrations in blood during their breeding season in the Antarctica. In the present study we investigate the biomagnification of PFAS in south polar skua from its prey, in two colonies with different prey availability; in the inland Dronning Maud land colony at Svarthamaren, the skuas prey almost exclusively upon Antarctic petrels (*Thalassoica antarctica*), of which the eggs and chick are preferred. In Adélie land, at the coastal colony near Dumont D'Urville (DDU), the skua diet is more diverse and include food from the sea as well as chicks of Adélie penguin (*Pygoscelis adeliae*). Of the analysed PFAS at Svarthamaren, only PFUnA was quantified above the detection limit in all matrices (blood, egg, stomach content) and species (skuas and petrels), although not in all samples. The levels increased from stomach contents of petrels, to petrel adults, and further to petrel eggs and chicks. Skuas had the highest levels of all the PFAS. The levels of other PFAS in skuas were in general lower at DDU than in Svarthamaren, and apart from the presence of branched PFOS at DDU, the pattern was comparable. The major differences were that in prey items, more PFASs were present above detection limit at Svarthamaren than DDU; PFDoA, PFTriA and PFTeA were present in Antarctic petrel and not in Adélie penguin. This difference is likely due to the resident nature of the Adelie penguins, whereas petrels migrate to, and maybe sometimes cross the Antarctic Circumpolar Current during winter. Thus, the migrating skuas from both colonies probably reflect the PFAS exposure outside of the Antarctic regions during breeding season. The higher diversity of PFASs present in petrels suggests that these birds as well have sources of PFAS outside the Antarctic continent.

TU322

Climate and productivity affect total mercury concentration and bioaccumulation rate of fish in subarctic watercourse

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Climate change is increasing temperature and precipitation in subarctic Europe. These changes are enhancing tree growth enabling intensification of forestry into previously inhospitable subarctic regions. The combined effects of climate change and land-use intensification extend the warm, open-water season in subarctic lakes and increase lake productivity. To assess the joint effects of climate and productivity on total mercury (THg) bioaccumulation in fish, we conducted a space-for-time substitution study in 18 tributary lakes of a subarctic watercourse forming a gradient from cold pristine oligotrophic to warmer mesotrophic and eutrophic lakes. Increasing temperature, precipitation, and lake productivity were predicted to elevate length- and age-adjusted THg concentrations, as well as THg bioaccumulation rate in muscle tissue of whitefish (*Coregonus lavaretus*), vendace (*Coregonus albula*), perch (*Perca fluviatilis*), pike (*Esox lucius*), roach (*Rutilus rutilus*) and ruffe (*Gymnocephalus cernua*). A positive linear relationship was observed between THg concentration and climate-productivity in vendace, perch, pike and roach. Higher climate-productivity values of the lakes also had a positive linear (pike and whitefish or u-shaped; perch and ruffe) relationship with THg bioaccumulation rate. Increased THg concentrations reveal adverse effects of warming climate and increasing productivity on fishes, whereas less distinct trends in THg bioaccumulation rate suggest more complex processes.

TU323

UV radiation effects the Diatom (Skeletonema sp.) physiological responses to ocean acidification

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Nowadays, the increasing CO_2 concentration in atmosphere associated with anthropogenic CO_2 emissions is causing the average pH of world's oceans declining at a rate of about 0,002 per year, which could lead to a potential drop of 0,3 to 0.4 units by 2100. As the H^+ concentration increases with CO_2 dissolution, H^+ releases can partially reverse the secondary dissociation reaction of the seawater carbonate system, leading to a decrease in carbonate ions ($H^+ + CO_3^{2-} \rightarrow HCO_3^-$). Therefore, typical changes linked to ocean acidification (OA) are increased partial pressure of carbon dioxide (pCO_2), H^+ and HCO_3^-, decreased concentrations of CO_3^{2-} and decreases in the $CaCO_3$ saturation state. These changes will lead to a decrease in the alkalinity of seawater, and the subsequebnt decrease in buffering capacity will increase the magnitude of short-term pH changes in the future. In addition, in the marine environment, organisms are not only facing ocean acidification but also exposed to other stressors such as UV radiation (UVR). However, there is still lack of knowledge about how the combined effect of UV radiation and OA will impact primary producers. The objective of the present study was to investigate the effects of CO_2 and UV radiation on the diatom *Skeletonema costatum* using a combination of genomic, functional and apical (adverse) toxicity endpoints. Small-scale CO_2 experimental systems (multiple stress chambers) were constructed to provide CO_2 levels of 350-1000 ppm pCO2 (pre-industrial, present day and future oceanic CO_2) and placed into UV exposure chamber (0.25 w/m^2). 72h exposure to elevated CO_2 caused and increase in *S. costatum* reproduction rate. At the cellular level, increased CO_2 reduced ROS formation, lipid peroxidation, carotenoids and increased chlorophylls (Chl *A* and Chl *B*) accumulation in *S. costatum*. There was no significant change in photosystem II performance after single CO_2 exposures. However, single UVR induced strong ROS formation, LPO and also reduced PSII efficiency and chlorophyll content after 72h exposure. When exposed in combination, enhanced effects on chlorophylls accumulation was observed. Additive effects were observed for oxidative stress, PSII performance and growth responses which indicated OA and UVR have independent modes of action in diatoms and there is no interaction between them under the present exposure scenarios.

TU324

Identifying further chemicals of emerging Arctic concern: A review of 'In silico' screening of chemical inventories

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The recent Arctic Monitoring and Assessment Program (AMAP) assessment of chemicals of emerging arctic concern included 150 individual chemicals and groups of substances reported in the arctic environment. However, these data represent just a tiny fraction the approximately 157,000 substances that have been registered for use in Europe, USA and Canada over the past 30 years. In the past 12 years many studies have screened lists of thousands of chemicals in the industrial chemical inventories of the European Union, the USA and Canada with the goal of identifying and prioritizing chemicals which are persistent (P), bioaccumulative (B) and toxic (T). A major goal of our study was to review this recent literature on computer-based or 'in silico' screening for POPs and assemble a list of possible target chemicals and then to develop a shorter list of possible priorities. We selected 8 studies published from 2066 to 2015 which had mainly utilized lists of industrial chemicals developed by chemical management regulators in Europe, USA and Canada and which are publically available. The combined list of chemicals identified by all studies contains 3425 substances after removing duplicates as well as Stockholm Convention POPs and was limited mainly to neutral organic chemicals for which existing QSPR models for predicting P, B and T are most applicable. The substances can be roughly categorized into eight classes; brominated, chlorinated, fluorinated, organophosphate esters, siloxanes, musks, polycyclic aromatics, and a broad class of non-halogenated organics.Phys-chemical properties and overall persistence (Pov), transfer efficiency (TE), and bioaccumulation factor (BAF) were calculated with the OECD Toolkit and POPs scores were calculated based on the sum of z-scores for logPov, logBAF, and logTE. The Arctic Contamination and bioaccumulation Potential (AC-BAP) was determined based on the following thresholds log Kow > 3.5, log Koa > 6, and 0.5> log Kaw >-7. Comparisons with recently updated chemical inventories showed that 316 of the 3425 substances were on the REACH inventory (1.9% of the 16755 substances with CAS RNs as of 2018) while 279 were on the US EPA Chemical Data Reporting for 2016 (3.2% of 8700 substances in active use in the US). Thus the results show that there are significant numbers of substances in current use or previously produced in large quantities which could be of concern in terms of transport and accumulation in the Arctic environment.

TU325

Environmental Risk Assessment in Antarctica: field actions, preliminary results and ongoing assays of the project ReACT

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Antarctica is the most remote continent on Earth. Due to its remoteness and harsh environment, Antarctica has been considered a pristine environment where human activities have very little impact. However, human presence in Antarctica has been rapidly increasing and human activities are known to have been changing the landscape and impacting the autochthonous biodiversity and ecosystems for at least the past half-century. In specific, preliminary studies have highlighted environmental contamination by major and trace elements and linked environmental hazardous potential in Fildes Peninsula, King George Island, Antarctica. The ReACT project capitalizes on these preliminary studies and on the recognised need for a better support of the major challenge of the Protocol on Environmental Protection to the Antarctic Treaty, i.e. the planning of human activities to minimize impacts upon the Antarctic environment and ecosystem. Site-specific Environmental Risk Assessment (ERA) is accepted worldwide as a reliable tool to appropriately frame decision making regarding the most adequate management towards environmental protection and/or remediation strategies. Taking this into consideration, a field campaign was held in February-March 2018 and we collected 20 soil samples in Fildes Peninsula (plus two likely references in Ardley Island and Nelson Island) for numerous analyses that will feed all the three Lines of Evidence (LoE) integrating ERA frameworks: the chemical LoE with soil physicochemical parameters, as well as trace element and polycyclic aromatic hydrocarbon contents; the ecological LoE with microbial community activity and structural diversity, as well as collembolan abundance; the ecotoxicological LoE with direct toxicity tests with soil organisms and tests with the bioavailable fraction of contaminants with freshwater organisms. So far, we completed the soil general physico-chemical characterisation. While soil density was found very similar among sampling sites, there are noticeable differences in pH, conductivity and organic matter content that may relate to existent gradients of contamination. Ongoing work includes chemical quantification and ecotoxicological testing with both Antarctic and ecotoxicological model species. Ultimately, the project ReACT aims to robustly integrate information from the three LoE to deliver feasible risk estimations for the focused area.

TU326

Snow buntings (Plectrophenax nivealis) as bio-indicators for exposure differences to legacy and emerging persistent organic pollutants from the Arctic terrestrial environment

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Eggs of snow buntings (*Plectrophenax nivealis*) were applied as a bio-indicator to examine differences in exposure to legacy persistent organic pollutants (POPs) and perflouroalkyl subtances (PFAS) from the Arctic terrestrial environment surrounding the settlements of Longyearbyen, Barrentsburg and Pyramiden on Svalbard, Norway. Significantly higher average concentrations of summed polychlorinated biphenyls (SumPCB$_7$: PCB 28/31, 52, 101, 118, 138, 153, 180) in eggs collected from Barrentsburg (350 ng/g wet weight (ww)) and Pyramiden (600 ng/g ww) compared to Longyearbyen (6.8 ng/g ww) are attributed to local sources of PCBs within these settlements. Average concentrations of Perfluorooctane sulphonate (PFOS) and several perfluorinated carboxylic acids (PFCAs) in snow bunting eggs were found to be statistically higher in populated settlements of Longyearbyen (PFOS: 3.8 ng/g ww; PFCAs: 13 ng/g ww) and Barentsburg (PFOS: 3.5 ng/g ww; PFCAs: 6.4 ng/g ww) compared to the abandoned Pyramiden (PFOS: 0.5 ng/g ww; PFCAs: 1.7 ng/g ww). Narrow foraging ranges of snow buntings during breeding season was useful in assessing point sources of exposure for PCBs and PFAS at particular sites with extreme differences observed between nest locations. SumPCB$_7$ concentrations ranged from 2 μg/g ww to below detection limits between nest sites located less than a kilometer from each other in Pyramiden. Similar findings were in Longyearbyen with several PFCAs from 2 to 55 times higher between nest sites with similar spatial distance. These findings indicate that snow buntings are a useful bio-indicator offering high spatial resolution for contaminant source apportionment in terrestrial environments on Svalbard.

TU327

Multiple exposure to legacy and emerging pollutants in populations of Boreogadus saida from inner and outer Bessel fjord (NE Greenland)

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NE Greenland is likely the last remote region of the Northern hemisphere - poorly known and understudied due to the limited access. This region is of high scientific interest, and value, as it harbour organisms with novel biological, ecological, and eco-toxicological features and adaptations. Reports by Arctic Monitoring and Assessment Program (AMAP) show an informational gap in regard to contamination in this area. Indeed, the list of chemicals found in Arctic ecosystems are continuously growing and for some current-use chemicals increasing temporal trends have been reported. Specimens of the marine gadoid *Boreogadus saida* were caught from R/V Helmer Hanssen in Bessel Fjord (BF), NE Greenland (76°N), in September 2017, during the scientific expedition TUNU-VII (UiT The Arctic University of Norway). The general aims were to evaluate the bioaccumulation of legacy end emerging contaminants in populations of *B. saida* from mid and outer Bessel Fjord waters, and from the continental shelf. Liver and muscle samples were analysed to determine the concentration of Polyaromatic Hydrocarbons (PAHs), Bisphenol A (BPA), Nonylphenols (NPs) and Organochlorine Pesticides (HCHs, Endosulfan, DDE, DDT) including some Current Use Pesticides (e.g. Chlorpyrifos, Dacthal). PLE extraction and analytical determination using LC-MS and GC-MS showed the occurrence of these contaminants in the majority of samples. Higher levels were detected in samples from the outer BF: BPA and NPs were 12.1 and 9.1 ng g^{-1} wet weight in muscle, values higher than those detected in liver samples that were 2.4 and 7.3 ng g^{-1} wet wt for BPA and NPs, respectively. Generally, higher concentrations of other contaminants occurred in the liver compared to muscle. The contaminant longitudinal pattern was outer BF > mid BF > shelf: Chlorpyrifos and Dacthal were 8.4 and 7 ng g^{-1} wet wt in the liver samples and 6.6 and 7.1 ng g^{-1} wet wt in the muscle, from of the outer BF. Results confirm the presence of selected legacy and emerging contaminants in this species, suggesting their long-range transport and the transfer of multiple contamination to the Euro-Arctic marine trophic webs.

TU328

High inputs and non-commercial polychlorinated biphenyl congeners in glacial surface snow from Svalbard: Are there new sources?

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In 2014 we collected surface snow samples from four glacial sites on Svalbard, Kongsvegen, Holtedaholfonna, Lomonosovfonna, and Austfonna, and analysed them for 209 polychlorinated biphenyl (PCB) congeners. There were 61 congeners below detection or contributed less than 0.2% to SPCB and were removed from the data sets, leaving 148 PCBs represented by 100 peaks. Two of the sites (Holtedahlfonna and Lomonosovfonna) are above the tropospheric boundary layer (BL) all year restricting atmospheric inputs from local sources, while Kongsvegen and Austfonna are within the BL during summer. The sites are within 220 km of each other. The SPCB fluxes (in pg cm^{-2} yr^{-1}) between sites are variable: Kongsvegen is 26.7 Holtedaholfonna 18.7, Lomonosovfonna 14.4, Austfonna 15.4. While these values vary by nearly a factor of 2, the air mass back trajectories (developed using HYSPLIT) are similar, all showing 40 – 45% frequency of air mass flow from expected source regions to the south and east, indicating that there may be local sources, particularly to the Kongsvegen site. All samples contain PCB-11 which was not produced in major PCB products but is likely a co-synthesis product of various yellow and orange pigments. PCB-5 was also observed in all samples and was not produced commercially in Aroclor (USA, UK) or Chlophen (Germany) products but may have been produced in the Russian PCB product "Trichlorodiphenyl". However, PCB-5 is also a co-synthesis product in manufacture of pigment violet (PV) 23. Considering that PCB-11 is common in Svalbard snow and ice samples, other pigment sources could be considered. Each of the four surface snow samples is dominated by PCB-52 which is different from a snow sample from Lomonosovfonna collected in 2010 which was dominated by PCB-70+74. PCB-52 is found in many commercial PCB formulations, however is not the dominant congener in any formulation that we investigated. What might be the source of this unusual amount of PCB-52? Investigation of the literature shows that PCB-52 is a co-synthesis product of pigment red (PR) 9, and perhaps other red pigments. Considering the somewhat unusual types and amounts of PCB congeners in these surface snow samples, investigation of unintentional PCB co-synthesis from production of non-PCB products must be hypothesized as a source of some PCB congeners to Svalbard.

TU329

Perfluoroalkyl substances in penguin eggs from the Ross Sea (Antarctica): evidence of 15 year of sampling

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PFASs have been detected in most ecosystems, including those in remote areas. They are long-range transported and can reach the polar regions. Antarctica and the Southern Ocean show extreme climate; due to the low temperatures and winter darkness, contaminants degrade very slowly; moreover, they can be trapped in snow and ice and eventually released during summer melting. When in the seawater, they can enter trophic webs and bioaccumulate mostly in predators that feed directly or indirectly on the cryopelagic community. PFASs include thousands of chemicals but the compounds usually detected in the environment belong to the class of perfluoroalkyl acids (PFAAs), such as perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA). The Stockholm Convention has recently restricted the production and use of PFOS; however, it is still manufactured and used in various industrial processes in China. Unhatched eggs are very useful non-destructive samples already successfully used to determine chemical levels; the POP transfer from mother to egg has already been reported too. The presence of contaminants in the egg is due to their transfer from the female during the ovogenesis process. Unhatched eggs of Adèlie penguin (*Pygoscelis adèliae*) (n=11) were collected at Edmonson Point (74°21'S, 165°08'E) (Ross Sea, East Antarctica) during the XVII (2001/02) and the XXX (2014/2015) Italian Expeditions, in the framework of the Italian Antarctic Research Programme (PNRA). The general aims were to assess the PFAA bioaccumulation in penguin eggs, to assess their transfer to the chick embryo, and to evaluate a temporal trend. On-line SPE-HPLC-MS revealed the occurrence of 12 PFAAs in eggs and chick embryos. PFOS and PFOA were detected in all samples collected in 2001, with concentrations ranging 0.3-0.53 ng g^{-1} wet weight (wt) in eggs (mean value: 0.42 ng g^{-1} wet wt) and 0.05-0.08 ng g^{-1} wet wt (mean value: 0.07 ng g^{-1} wet wt) in chick embryos. Lower levels were detected in 2015 samples, with mean values of 0.3 and 0.005 ng g^{-1} wet wt of PFOS and PFOA, respectively. This decreasing trend was not observed for PFHxA, PFNA, PFDA, PFUnA, PFDoDA, PFTrDA, PFTeDA concentrations. PFHxA showed the highest levels in the egg samples collected in 2015 (mean value: 0.8 ng g^{-1} wet wt), confirming an ongoing input of these compounds from long-range transport and/or local sources.

TU330

The relationship between per- and polyfluoroalkyl substances (PFASs), persistent organic pollutants (POPs) and trace elements and thyroid hormone status in glaucous gulls (Larus hyperboreus)

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The aim of this study was to investigate the concentrations of per- and polyfluoroalkyl substances (PFAS), persistent organic pollutants (POPs) and trace elements and their relationship to the levels of thyroid hormone (TH), thyroid stimulating hormone (TSH) and thyroid gland histology in glaucous gull (*Larus hyperboreus*) from Svalbard. The glaucous gull is an apex predator in the Arctic ecosystem. A total of 35 glaucous gulls were captured and euthanized in the area close to Longyearbyen, Svalbard, in the spring of 2017 and 2018. The level of POPs and PFASs were measured in liver, and trace elements have been measured in plasma, feathers, liver, muscle and kidney. The levels of free and total triiodothyronine (T3) and thyroxine (T4) and TSH were measured in plasma. THs are crucial in the regulation of several important physiological processes such as growth, development, reproduction, thermogenesis, molting and behavior. Organic chemicals, elements or other factors that disrupt the THs system can therefore potentially cause severe effects on individuals and populations. It is therefore important to investigate the combined effect of potential TH disruptors in wildlife. Association between THs and POPs and PFAS have been identified in glaucous gull, but neither the relationship between elements and THs, or between TSH, TH, pollutants and thyroid gland histology have previously been reported. In the present study, were the concentrations of perfluorooctanesulfonic acid (PFOS) and the long chained perfluorinated carboxylic acids (PFCAs) high while the short chain PFCAs and emerging PFASs were not detected or had low concentrations. The levels of PCBs and certain pesticides were high, while the levels of PBDEs were low and new brominated flame retardants were not detected. Of the elements the levels of Cd were high and the levels of Zn, Hg and Se were considerable (concentrations were compared to other Arctic seabirds). The preliminary results indicate that PFASs and Hg:Se ratio were correlated to the TSH levels and that Cd was associated with the level of TT3, FT4 and TT3:FT3. The level of Zn was associated with the level of TSH and TT3. Follicle count in the thyroid gland was positively correlated with Hg and showed a possible relationship to the levels of PFAS but the results were inconclusive. The relationship between POPs and the thyroid variables need to be further investigated. The project is funded by The Research Council of Norway, no 268419.

TU331

Influence of Permafrost Disturbances and Increased Turbidity on Trends of Mercury and other Elements in Arctic Char in East and West Lake, Melville Island, Nunavut

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The Cape Bounty Arctic Watershed Observatory (CBAWO) on Melville Island in the Canadian Arctic Archipelago, includes two adjacent, geologically similar watersheds, West and East, which are currently undergoing climate-driven changes. Climate over the period 2007-2012 was unusually warm during summer months and resulted in changing hydrology and permafrost degradation across the area. In addition, West Lake experienced subaqueous slumps over this period, which may also be related to permafrost thawing along the shoreline. The East Lake catchment has experienced only minor permafrost disturbances, and no change in turbidity. We are investigating whether these changes are also seen in bioaccumulation of mercury (Hg) and other contaminants in landlocked arctic char (*Salvelinus alpinus*) and the food webs of West and East Lakes. Char have been collected annually at the end of July, from 2008 to 2018 (except for 2010), and analysed for a suite of 34 elements using ICP-MS, and Hg usingUSEPA Method 7473 with a Direct Mercury Analyser. Carbon (C) and nitrogen (N) stable isotope analysis showed that char have significantly more depleted $\delta^{13}C$ in adult char (>200 g) in East vs West Lake (mean ± SD; -27.27±0.81 ‰ (N=98) vs -24.73±1.17 ‰ (N=97)) indicative of greater terrestrial and benthic carbon inputs to West Lake. The combined results from 2008 to 2018 collections show that the West Lake adult char have significantly higher concentrations of Hg (geomean/range = 0.146 (0.036-0.581 ug/g wet wt)) compared to East Lake (0.079 (0.023-0.285 ug/g wet wt)) and this difference is even greater if results are adjusted for $\delta^{15}N$ or length. Condition factors (g*100/cm^3) for adult char in West Lake have declined since 2008 and over the period 2011-2018 have been significantly lower (0.62±0.12) than those in East Lake (0.66±0.09) indicating they are thinner than fish of the same length in East Lake. This may be due to difficulty feeding in West Lake's turbid waters. Hg concentrations have declined steadily in East Lake char over the period 2008 to 2018 (averaging -6.7 %/yr) while increasing (5.3 %/yr) in West Lake from 2009-2018. The higher concentrations and increasing Hg in West Lake char are consistent with higher inputs into West Lake resulting from extensive permafrost disturbance in the watershed as well as from higher suspended particulate concentrations in lake waters due to the turbidity

TU332

Do population parameters influence the role of seabird colonies as secondary pollutants source? A case study for Antarctic ecosystems

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Antarctica is home to a large avifauna of both endemic and migratory bird species. Recent studies, including some by the authors of the present work, confirmed the role of seabird colonies in Antarctic ecosystems as relevant secondary sources of Persistent Organic Pollutants (POPs) and Trace Elements (TEs) as well. Birds undergo bioaccumulation and biomagnification of these pollutants throughout their lives and, during their reproductive period, they gather in large numbers into large colonies and excrete and die on land. This represents a local source of not only nutrients to terrestrial and possibly coastal marine ecosystems, but also of POPs and TEs. When comparing the data obtained within our previous works and published literaturen most cases, colonies of birds of higher trophic level (therefore more prone to biomagnification), generally small and sparse did not present an influence as stark and as frequent as the large and dense colonies of birds with a lower trophic level. By means of a cross examination of the datasets of two papers of the authors verifying the role of the colonies as secondary sources of POPs and TEs and also census data of the very same spots, we could explore the influence of population parameters and not only the trophic level of the species. Preliminary results obtained by correlation analyses and Generalized Linear Model building and ranking by the corrected Akaike Information Criterion (AICc) suggest that the total population parameter is of equal or more importance than the previous categorical (location, colony species, matrix species) and continuous ones (%C, %N, $d^{13}C$ and $d^{15}N$) in several cases: for Hg in soil, for Cd in mosses and soil, and for PCBs in mosses. Interestingly, the population parameter was not present in the most accurate models for lichens, likely due to the fact that most of the analytes of interest are volatilized and not absorbed by the lichens at the same rate as the organic matter originated from the colonies. The dynamic parameters of the populations are yet to be included in the analyses and might also shed a light on the issue in the upcoming publication. Yet, as both population parameters and dynamics can be affected by climate change, some indirect effects could be hypothesised in the near future based on our results.

TU333

Temporal trends of persistent organic pollutants in Barents Sea polar bears (Ursus maritimus) in relation to changes in feeding habits and body condition

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Temporal trends of persistent organic pollutants (POPs: PCBs, OH-PCBs, *p,p'*-DDE, HCB, β-HCH, oxychlordane, BDE-47 and 153) in relation to changes in feeding habits and body condition in adult female polar bears (*Ursus maritimus*) from the Barents Sea subpopulation were examined over 20 years (1997-2017). All 306 samples were collected in the spring (April). Both stable isotope values of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) from red blood cells declined over time, with a steeper trend for $\delta^{13}C$ between 2012 and 2017, indicating an increasing intake of more terrestrial and lower trophic level prey. Body condition, based on morphometric measurements, did not significantly change between 1997 and 2005, and increased significantly between 2005 and 2017. BDE-153 and β-HCH concentrations in plasma were stable over time, whereas Σ_4PCB, Σ_5OH-PCB, BDE-47 and oxychlordane declined linearly. Plasma concentrations of *p,p'*-DDE and HCB, however, declined until 2012 and 2009, respectively, and increased thereafter. Climate-related changes in feeding habits and body condition did not significantly affect POP trends. The study indicates that changes in diet and body condition were not the primary driver of POPs in polar bears, but were controlled in large part by secondary and/or possibly primary POP emissions.

Human Health and Environmental Risk Assessment of Chemical Mixtures: Moving Towards the Non-toxic Environment (P)

WE001
Considerations to modify "WHO/IPCS framework on risk assessment of combined exposure to multiple chemicals" for regulatory environmental risk assessment
K. Yamazaki, Ministry of the Environment / Environmental Health
The goal of our study on mixtures assessment is to develop the way to introduce "assessment of combined exposure to multiple chemicals" into environmental risk assessment practices for regulatory objectives, such as selecting high-priority chemicals, setting criteria/standards, or identifying chemicals to be regulated. The WHO/IPCS Framework on risk assessment (RA) of combined exposure to multiple chemicals would be useful for regulatory approach. We have been considering how we should adapt the framework to our RA practices, from screening-level to comprehensive ones, through case studies on environmental RA for some groups of chemicals, such as acrylates and phthalates. Two elements could be included into the Framework for regulatory application. [1] Prior to the tiered risk assessment, the Framework indicates Problem Formulation with rationale to consider combined exposure. In the chemical-by-chemical environmental RA for nationwide regulatory objectives, target chemicals have been selected based on expected or potential environmental risks. Screening-level RA of combined exposure from multiple chemicals should be considered in the similar context. Initially one or a few target chemicals would be selected, without enough information for appropriate problem formulation. Next question should be "Could risk assessment be conducted for the certain chemicals only? Or should any other chemicals with similar structure be considered for risk assessment as well?" Reasonably conservative consideration should be included in the Framework, so as not to overlook false negatives with limited/insufficient information. [2] The Framework, which was designed for human health RA, cannot be applied to regulatory environmental RA as it is. Tiers in the Framework can be correlated with respective stages of the existing regulatory RA practices, where PNEC values have been derived referring to ecotoxicological studies. Characterization of common effects might not necessarily be practical for ecotoxicity data, where knowledge on mode of action cannot always be available or identifiable. Instead we might have to be satisfied with derivation of relative potency factors (RPFs) by just knowing similarities/tendencies of ecotoxicity values. These modification should be included into the Framework. Detailed considerations to modify WHO/IPCS framework for regulatory environmental risk assessment will be presented at the Meeting.

WE002
From mapping to identification: Paving the way to UVCB risk assessment
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Risk assessment of complex substances such as multi-constituent substances, and substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs), presents numerous challenges. International regulatory programs (specifically REACH, Canada's Chemicals Management Plan, and USEPA's PMN process) have highlighted the complexities of characterizing fate and exposure, risk assessment, and registration of these types of substances. This is why HESI's UVCB Committee is working on an approach to assess ecological risks of UVCBs, and meet regulatory needs. First, a framework was created to map the vast and complex universe of UVCB substances according to chemical and/or functional classes, exposure and use patterns, production volume, potential toxicity and other criteria. This grouping system is the foundation of all work performed on this project, by providing a better understanding of the nature of these substances and their sector of use, as well as some insight on which substances present the most significant regulatory challenges. In a second step, the Committee will develop identification and characterization strategies for the mapped substance groups. For each group, the overall goal is to determine the minimum level of information required to perform a robust and fit-for-purpose ecological risk assessment. To do so, the Committee has been exploring various issues for given substance categories, including definitions of acceptable levels of uncertainty and variability to assess risks; strategies to characterize substances with limited or no structural information; and development of criteria that could be used in a weight of evidence approach. Characterization approaches have included discussions around how knowledge of source material and production processes can help inform substance identification. This two-pronged approach is expected to ensure that the efforts and resources deployed for UVCBs risk assessment match actual needs, and help streamline the risk assessment process. Examples of UVCB mapping will be presented, and some of the most common categories of UVCBs will be highlighted along with the associated assessment challenges. Substance identification and characterization methods will be illustrated, as well as how they provide the necessary information for a reliable risk assessment.

WE003
Environmental Risk Assessment of Technical Mixtures under REACH - a regulatory view on the LCID methodology
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Chemicals are usually processed and end up in mixtures. Still, the REACH regulation focuses on single substances and their safe use conditions, while in other substance regulations mixtures are partly addressed during product authorization. For REACH an environmental risk assessment of mixtures is challenging: Manufacturers or importers of chemicals into the EU are responsible for registering their substance and provide information for intrinsic substance properties, information on fate and behavior, and hazards and risk. The registrant is required to assess the intended uses and processing, but lacks often information to assess the safe application during the entire lifecycle. The downstream users know the details on formulation and processing of their mixture(s) or product(s), but only have basic data to adapt the environmental risk assessment or derive the safe use amounts. REACH addresses the safe use of single substances in technical mixtures, but not explicitly the possible risks arising from joint effects and exposures of components in mixtures (Art. 10 of the REACH Legislation). From the regulatory point of view it is important to tackle mixtures already at the "source" concerning their uses and possible technical mixtures, before substances enter environmental compartments regardless of their common impact. Assessment and regulation of these common impacts are only possible at this level. The safe use for technical mixtures has to be ensured. Indeed, REACH actors have legal obligations (Art. 33 of the REACH Legislation) to ensure safe use of articles and substances, also substances in mixtures. Recently, some attempts have been made by industry organizations with the concept of LCID to improve the assessment and communication of safe use conditions for technical mixtures. However, essential improvements are needed with respect to data availability, quality and communication, the selection of substances to be considered during an assessment, training and guidances for downstream-user. Until now, LCID seems to be rather a prioritization and communication tool than providing a complete environmental risk assessment of chemical mixtures. Downstream user need training and support by guidances or also alternative methodologies if a derivation of safe use conditions is impossible due to scarce data. We will highlight the need to further develop mixture assessment under REACH.

WE004
Chemical activity as a unitless measure to assess the impact of chemical mixtures on the environment
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Aquatic sediments in river beds, lakes, and the oceans serve as a sink for many organic chemical pollutants that are persistent, bioaccumulative, and toxic, thereby posing long-term health threats towards the environment and humans. Microbial communities play an essential role in controlling the transformation and mineralization of organic compounds, rendering these consortia as suitable model systems to study the environmental impact of contaminants. Most studies focus on single pollutant effects, but knowledge about the impact of chemical mixtures is scarce. The two main reasons are: 1) measuring concentrations of all potential contaminants in the environment is unrealistic due to the technical challenges and analytical costs, and 2) the sum of the measured concentrations of different chemicals does not inform us about the exposure levels, because toxic concentrations are compound-specific. To overcome these constraints, we will investigate the effect of chemical mixtures on microbial communities using the

concept of ‘chemicalactivity’,a unitless proxy for quantification of chemical exposure in non-ideal solutions. This approach allows integratingexposure to organic contaminant mixtures using a combination of concentration and chemical solubility as a measure of chemical saturation in the system. In experiments, we will expose microbial communities to chemical mixtures, with the concentration of each chemical below the threshold level of its specific toxicity. The latter will ensure that the observed effects are not related to the specific mode of action of the test chemicals present in the mixture. Total community activity metrics, such as growth and respiration, will be used as endpoints. Moreover, to evaluate possible biogeochemical responses to the exposure, we will analyse functional gene abundance in the community. These experiments will help to understandand assess the resilience of sediment communities towards chemical pollution as well as to develop useful community-level proxies.

WE005

Predicting ecological changes downstream of wastewater outfalls

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In many regions of the world, untreated wastewater is discharged directly into rivers containing sanitary determinands including ammonia, nitrite and organic matter which places a demand on dissolved oxygen in the water. The wastewater may also contain chemical ingredients of home and personal care products. When sewage treatment is lacking, often in developing regions, these sanitary determinands and down-the-drain chemicals could be present at concentrations in surface waters which may adversely impact the ecological communities present downstream of the effluent outfall. Some studies have investigated these ecological effects by sampling the taxa present at regular intervals downstream of a wastewater outfall, from which a common pattern in terms of macroinvertebrate species richness, dominance and diversity throughout the impact zone is evident. The aim of this work was to develop a model to predict the ecological composition downstream of an effluent outfall, as a result of the concentration gradients of multiple stressors. The model combines water quality data and toxicity data of the stressors on aquatic organisms, in the form of species sensitivity distributions (SSDs) to predict this impact against the exposure of the stressors. The model was based on selected stressors: ammonia, nitrite and dissolved oxygen which are present, in particular, in untreated wastewater; two chemical ingredients used in home and personal care products (LAS and triclosan) which are washed down-the-drain. First order rate kinetics were applied to predict the stressors’ decreasing concentrations, while the varying dissolved oxygen concentrations were predicted using the Streeter-Phelps model. The model was applied to measured data from a field study on the South Elkhorn Creek in Kentucky, USA. Predicted effects on taxonomic composition were in line with field observations, although further enhancements to the model could incorporate more environmental realism. This was a useful step in the direction to creating a model of the impact zone ecology in rivers.

WE006

Risk-based prioritization of multiple metals in seafood among children and teenagers and implications for food safety management

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Human health risks associated with the consumption of metal-contaminated seafood have received continual public concern. Multiple metals simultaneously accumulate in a variety of seafood and therefore pose mixture health risks to humans. Given the multitude of potential foodborne hazards, limited resources should be focused on the greatest risks among the many metals and seafood products. The RISK21 visualization methodology, developed by the Health and Environmental Sciences Institute, is a useful tool in priority setting. Therefore, the major purpose was to apply the RISK21 methodology to compare and rank health risks posed by multiple metals through consumption of seafood products among children and teenagers in Taiwan for identifying which metals and seafood products should be of priority concern. The concentrations of metals in a variety of seafood sold in markets were obtained from the Taiwan government. Using data from the Nutrition and Health Survey in Taiwan, we quantify the consumption rates of various seafood products for toddlers (0 – 3 years), preschoolers (3 – 6 years), children (6 – 12 years), and teenagers (12 – 18 years). The possible joint toxic actions of metal mixtures were characterized mainly based on toxicity interaction information available from the Agency for Toxic Substances and Disease Registry. We found that the mixture of inorganic arsenic (iAs), cadmium (Cd), lead (Pb), and methylmercury (MeHg) cause neurological toxicity. The RISK21 shows that the iAs and MeHg in saltwater fish as well as Cd and Pb in shellfish were listed as high priority for food safety management for children of all ages. Moreover, Pb in saltwater fish and Cd in cephalopods were listed as high priority for toddlers and teens, respectively. We thus suggest that regular monitoring of iAs and MeHg in saltwater fish as well as Cd and Pb in shellfish and cephalopods are warranted. Our work helps to identify metals in several types of seafood products that warranted the regular monitoring, allowing risk manager to design the risk-based monitoring program and the possible strategies for food safety management.

WE007

Mixture risk assessment and prioritization of multiple aldehydes in fried foods

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Low molecular weight aldehydes are produced during the deep-frying produces as a result of the dehydration of glycerol. Many of the aldehydes have been considered the mutagenic compounds associated with the genotoxicity, carcinogenicity, and oxidative stress, including formaldehyde (FA), acetaldehyde (AA), and acrolein (AC). The purpose of this study was to (1) assess the mixture health risks of co-exposure to FA, AA, and AC through dietary intake of fried foods for different age populations in Taiwan and (2) to identify which fried foods pose the greater dietary exposure risk to each age population through risk prioritization. In this study a total dietary study-like investigation was conducted to design a sampling list of fried foods that most commonly consumed by Taiwanese. Concentrations of FA, AA, and AC in fried foods were analyzed using the headspace solid-phase micro-extraction-gas chromatography-mass spectrometry. Hazard index (HI) was used to characterize the mixture risks posed by the dietary intake of FA, AA, and AC from fried foods. Moreover, the RISK21 approach was applied to perform risk ranking. The concentrations of FA, AA, and AC in fried noodles were 3.26 (95% CI: 1.59–5.89 mg/kg), 33.08 (8.21–92.24 mg/kg), and 1.62 mg/kg (0.49–4.07 mg/kg), respectively. For potato chips, the levels of FA, AA, and AC were 2.43 (1.21–4.90 mg/kg), 18.86 (5.94–61.05 mg/kg), and 1.91 mg/kg (0.66–5.59 mg/kg), respectively. Risk analysis showed that children had the highest risk of gastrointestinal tract damage, with 97.5th percentile HI estimate of 4.16. For teenagers, adults, and elderly persons, HI estimates were all less than one. RISK21 analysis indicated that health risks related to the consumption of fried noodles were moderate concern for children. Our analyzes indicate that the health risk associated with dietary exposure to multiple aldehydes in fried foods is unlikely to occur for most populations but is a potential health concern for children.

WE008

Accumulation of perfluoroalkylated acids (PFAAs) in Belgian home-produced chicken eggs along different directions from a fluorochemical plant

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Perfluoroalkylated acids (PFAAs) can enter the food chain due to their persistence, widespread use and global distribution. Field research has demonstrated that these substances can bioaccumulate and biomagnify in wildlife. Hence, it is very plausible that PFAAs can biomagnify to high concentrations in humans. For these reasons, PFAAs may pose a significant risk to human health as residents may inadvertently consume PFAA contaminated food. Over the last decade, humans consuming food products from self-cultivated origin has become a remarkable trend in rural, urban and even industrial environments. For instance, the housing of free-ranging chickens for egg production has gained popularity as eggs constitute an important component of the Western-European diet. At the same time, the dominant exposure pathway of PFAAs is the dietary intake route. Therefore, the main objective of this study was to analyze and examine the pattern of PFAAs in home-produced chicken eggs from households and to assess potential health-risks of PFAAs via consumption of these eggs to local residents. In total 70 individual eggs were collected from 35 volunteers who kept free-ranging laying hens within a radius of 5 Km from a fluorochemical plant in Antwerp. In total, nine PFAA compounds could be detected in the egg samples. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were the two most frequently detected compounds with equal detection frequencies of 97%. The concentrations of these compounds ranged from, respectively, 0.54 – 241 ng/g ww and 0.21 – 2.38 ng/g ww. PFOS concentrations followed a clear decreasing pattern from the fluorochemical plant onwards, which indicates that ingestion of soil particles and organisms through pecking behavior might be a dominant source of PFAA contamination in the eggs. PFOS concentrations in some of the eggs exceeded the tolerable daily intake (TDI) values. This study demonstrates that the consumption of home-produced eggs can be an important dietary exposure pathway of PFAAs to humans, although the concentrations mostly did not exceed the available tolerable daily intake values. Finally, PFOS may represent a possible health-risk to local residents via the consumption of home-produced eggs.

WE009

BEA (biomonitoring in adolescents) study protocol: design of a national human biomonitoring survey in spanish adolescents

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In 2016, the Ministry of Agriculture, Food and Environment (MAPAMA) funded the BEA (biomonitoring in adolescents) study, the first nationwide human biomonitoring survey carried out in Spain to study the levels of selected environmental pollutants in urban Spanish adolescents (14-16 years old). The different step needed to develop this human biomonitoring study, both field work preparation and the sampling campaign development will be presented. In summary, adolescents of both gender, were recruited through twenty secondary school centers from 11 cities with more than 150000 inhabitants. These cities were selected taking into account their distributed throughout the entire Spanish territory, prioritizing those in which no similar studies had been carried. The environmental pollutants include in BEA Study were metals: urinary mercury, total mercury in hair and urinary cadmium, as well as organic plasticizer additives such as Bisphenols and phthalates metabolites in urine. Sampling was carried out from October 2017 to February 2018, and it included the sample collection of hair and urine that were mandatory matrices. In addition, blood sample for future studies on persistent organic pollutants was taken as voluntary matrix. A self-administered epidemiological questionnaire was completed by all participants to compile individual information on lifestyle, environment and diet in order to assess potential exposure determinants related to the pollutants studied. The sample thus obtained had a total number of 498 adolescents. Acknowledgments: This work has been funded by MAPAMA and ISCIII

WE010

Adding up apples and pears: Mixture toxicity in environmental and human health pesticide risk assessment

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Often it is stated that current risk assessment of pesticides insufficiently addresses mixture toxicity. To help understand the coverage in current risk assessment guidance for human health and the environment, we present the current approaches, their commonalities and differences in an orchard case study and make recommendations how to align and simplify them. Consumer (dietary) risk assessment: Combined risk assessments for those active substances present in the same (mixed) formulation can be performed in a straightforward manner, using a tiered approach that assumes dose addition and can focus on compounds with similar toxic effects. Non-dietary risk assessment: In order to ensure a safe use of plant protection products for farmers handling the products (operators), workers re-entering a treated field, or bystander and residents who are only occasionally present in adjacent areas during or after pesticide use, combined risk assessments have to be presented for all active ingredients present in one product, assuming dose addition. Hazard quotients of the single active ingredients of the formulation are simply summed up to obtain a hazard index for the mixture. Environment: For birds and mammals, to the recent past, the formulation was tested in acute oral LD50 studies on birds (usually the Bobwhite quail) and mammals (usually the rat). Under EU regulation 1107, to reduce vertebrate testing, calculation methods are normally employed to predict the mixture toxicity of formulations. Two calculation methods (for birds, LD50mix according to EFSA GD B&M 2009, for mammals ATEmix according to CLP regulation 1272/2008) are conceptionally similar; however there are also some differences. For long-term risk assessments, no straightforward approach is commonly used, except for the TERmix in some EU central zone member states. For aquatic species, usually acute studies with the a.s. and with the formulation are available. In order to conclude on similarity, calculated mixture toxicity (ECxmix-CA) is compared against measured formulation toxicity (ECxPPP). Deviations exceeding factor 5 in either direction need to be addressed. In case of overall low toxicity or in case of a clear toxicity driver (a.s. contributing >90%) the assessment can be finalized. If a higher tier assessments is needed, the respective risk quotients (RQ) of each a.s. are added to calculate an RQmix, identical to (but differently named than) the hazard index in consumer risk assessment.

WE011

Risk Assessment of Pesticide Residue in Fish Samples From Donga River; health impact on consumers

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The use of obsolete or banned pesticides in most developing nations gives more reasons to worry because of the health of consumers of agricultural and fish products. In this study, the levels of the pesticide residues (DDT, DDD, Dieldrin, Aldrin, dicofol, methoxychlor, endosulfan, chlordane, heptachlor, pentachlorobenzene, mirex, toxaphene, hexachlorobenzene, α-HCH, β-HCH, and γ-HCH) in river Donga, Taraba State, Nigeria were assessed using fish samples from Donga river as a case study. Five fish species (*Synodotis membranaceus*, *Protopterus annectens*, *Clarias gariepinus*, *Heterotis niloticus* and *Tilapia zilli*) were collected for from the river for possible analysis for pesticide residues. The result showed that α-HCH, β-HCH, and γ-HCH were the most abundant pesticide residues in the fish samples. The result also showed that levels of α-HCH, β-HCH, and γ-HCH in *Synodotis membranaceus cupside* were above the maximum residue limits (MRLs). Therefore the health of consumers may be at higher risks when exposed to fish products from Donga river.

WE012

Elevated blood metal levels and cytogenetic damage induced in rats exposed in-situ to underground water and ambient air emissions at a municipal landfill, Nigeria

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Solid waste landfills in developing nations have been known to be major source of environmental pollutants including heavy metals, thus, constituting ecological risk to exposed populations. This study herein investigated the levels of lead (Pb), cadmium (Cd), chromium (Cr) in blood and potential cytogenetic alterations in rats exposed *in-situ* to underground water and ambient air emission from Olusosun landfill in Lagos, Nigeria. Male Wistar rats (5 rats/point/duration) were exposed at three different points to air emissions and underground water (via drinking) from the landfill for 4, 8, 12, 16, 20 and 24 weeks. Rats concurrently sited at 17.3 km from the landfill site served as control. Rats' blood levels of Pb, Cd and Cr were measured after each exposure periods using atomic absorption spectrophotometer (AAS) and formation of bone marrow micronucleus and polychromatic erythrocyte/normochromatic erythrocytes (PCE/NCE) ratio were analysed. The strength of the linear relationship between blood metal levels and cytogenetic alterations were determined using Pearson's correlation analysis. Blood levels (in mg/L) of Pd, Cd and Cr in the landfill exposed rats were significantly elevated ($p < 0.05$) compared to those of the corresponding control throughout each periods of exposure. Also, there was significant ($p < 0.05$) increase in the frequency of micronucleated polychromatic erythrocytes (MNPCE) with significant ($p < 0.05$) decrease in PCE/NCE ratio at all points and periods of exposure compared to the control. No strong correlation ($p > 0.05$) was observed between blood metal levels and exposure periods; frequencies of MNPCE; and PCE/NCE ratios respectively during the study periods. The concentrations (mg/L) of heavy metals such as Pb, Cd, Cr, Cu and Fe in the underground water were observed to be above permissible limit for drinking water. *In-situ* exposure to underground water and air emissions at the landfill led to elevated blood levels of heavy metals in exposed rats. However, no strong correlation observed between blood metal levels and cytogenetic damage implies that other unanalyzed toxic agents present in the landfill compartments might have contributed either additively or synergistically with the metals in inducing the observed cytogenotoxicity in the erythropoietic stem cells. This finding is of ecological and health risk to animal and human populations who co-exist in the vicinity of major municipal waste dumpsites in developing countries.

WE013

Assessing health risk from mixture exposure to ethanol and acetaldehyde through drinking alcoholic beverages

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Previous studies have explored the health risk associated with alcohol consumption; however, their studies have exclusively examined single ethanol (ETOH) or acetaldehyde (ACE) and have not yet fully considered the consider alcohol metabolism. Individuals with ALDH2-deficiency are unable to metabolize acetaldehyde, resulting in toxic ACE accumulating in the body. The prevalence of ALDH2*2 variant in Taiwanese is as high as 45–47%. Thus, this study aims to investigate the health risks of ETOH and ACE posed by drinking alcoholic beverages for adult Taiwanese with different ALDH2 genotypes. This study calculated daily intakes (DIs) of ETOH and ACE by multiplying the probability distribution of daily consumption of three types of alcoholic beverages (beers, wines and spirits) with the corresponding probability distribution of ETOH and ACE concentrations. By using data from the National and Health Survey in Taiwan, we estimated the consumption rates of different alcoholic beverages for adult Taiwanese. A previously well-developed human PBPK model with genetic polymorphism of ALDH2 was applied to quantify simultaneously the internal doses of both ETOH and ACE in liver. According to the available epidemiological and animal studies, we used benchmark dose modeling and the human equivalent dose approach to estimate point of departure (POD). We then converted PODs to corresponding internal dose metrics using human PBPK model. The concept of margin of internal exposure (MOIE) was used to assess the health risks of ETOH and ACE posed by drinking alcoholic beverages. We found that drinking spirits contributed highest DI estimates of ETOH and ACE for adult Taiwanese. Based on human epidemiological data, the estimated $BMDL_{1.5}$ was 35.73 mg/kg/day. Because of the lack of human data of ACE, the HED associated with animal NOAEL was estimated to be 21.88 mg/kg/day for hepatotoxicity. An acceptable MOIE for an internal dose-based risk assessment was considered 10 (interspecies difference). MOIE estimates of ETOH for individuals with any ALDH2 genotype were less than 10, indicating a high potential risk for liver damage from alcohol consumption. The MOIEs of ACE for individuals with ALDH2*2/*2 genotype

ranged from 68.73 to 5.02 10^5, indicating a low health concern of hepatotoxicity. We suggest that the priority action of alcohol policy for government was to reduce ETOH intake rather than to focus on ACE.

WE014

Probabilistic integrated PBPK model to assess health risk of dietary exposure to acrylamide

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Acrylamide (AA) is formed from numerous carbohydrate-rich foods during heat treatment above 120 °C as a result of Maillard reaction between amino acids, in particular asparagine, and reducing sugars. Lots of *in vitro* and *in vivo* studies have indicated that AA and its metabolites may lead to neurotoxicity, reproductive and developmental toxicity, genotoxicity, and multi-organ carcinogenicity. The major purpose of this study was to integrate probability and physiologically based pharmacokinetic (PBPK) model to assess the potential health risks of AA in 6 different foodstuffs (twisted cruller, brown sugar, potato chips, French fries and hash browns, coffee, as well as cereal and seafood snacks) for adults in Taiwan. Dietary intakes (DIs) of AA were calculated by multiplying the probability distribution of daily consumption of 6 types of foodstuffs with the corresponding probability distribution of AA concentrations. According to the available animal studies, a $BMDL_{05}$ for neurotoxicity was 0.27 mg/kg/day. We then use PBPK modeling approaches to convert external animal $BMDL_{05}$ and human DIs into the corresponding animal and human internal dose metrics. The concept of margin of internal exposure (MOIE) was applied to compare internal dose metrics for assessing the level of health concern of AA for neurotoxicity. A novel approach, termed Risk Assessment in the 21st Century (RISK21), was used to visualize the results of MOIEs and to perform risk ranking for identifying which foodstuffs pose greater dietary exposure risk to consumers. We found that the highest concentration of AA was brown sugar followed by potato chips, whereas coffee had the lowest AA contents. Our analysis results showed that snacks contributed highest DI estimates of AA for adults in Taiwan. Both of the developed animal and human PBPK models can well predict the urinary metabolites of AA (AAMA) and GA (GAMA). The internal dose-based MOIE approach could reduce the uncertainty to 25 (2.5 for interspecies differences in toxicodynamics and 10 for human variability in toxicokinetics and toxicodynamics). Results of the RISK21 showed that health risks of AA in 6 different foodstuffs were low concern. The proposed methodology in this study facilitates to make uncertainties explicit and to clarify the potential health risk of AA in thermally-processed common foods for adults.

WE015

The Toxic Potentials assessment and mitochondrial functions alteration of disinfection byproducts by Human Embryonic Kidney (HEK293) Cell Model

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Disinfection byproducts are inevitably generated during drinking water disinfection processes, and their hazards have not been well characterized. Due to the key role of kidney in fluid-electrolyte balance and detoxification of some xenobiotic, it is predictable that DBPs may do toxicological and pathological damage to human kidney. The human embryonic kidney (HEK293) cell was selected, instead of the commonly reported CHO cell, as a model to investigate the toxic potential and target of different kinds of DBPs. Based on the chronic toxicity parameter EC_{10} of the 24 hour-cell viability-test, we obtained the different toxic ranks of the tested DBPs (TBAL > CDIM > CAM > DBIM ≈ DCIM > BCIM > DCAM ≈ TCAM > TCAL) with previous studies and calculated their risk quotients by combining EC_{10} with chemicals' actual concentrations in drinking water systems. The rank of potential risk quotients for tested DBPs is: DCAM>TCAM>CAM; TCAL>TBAL(N/A); BCIM>DCIM>DBIM>CDIM>BDIM(N/A). Then, mechanism-related bioassays were performed in three representative agents (DCAM, TCAL, and BCIM) from 3 classes of DBPs. The levels of extracellular LDH and intracellular ROS were increased by the exposure of 3DBPs. In addition, significant apoptosis was induced by 10-1000μg/L DCAM/BCIM-treatment and the genes expression involving mitochondrial functions in apoptosis (*CASPASE-3/7/8* and *BCL-2* mRNA) and energy metabolism(*COX17* and *SLC25A6* mRNA) were correspondingly changed by 3 compounds exposure. All results suggested the effects of 3 DBPs interrupting the molecular, physiological and biochemical processes relevant to mitochondrial functions, such as oxidative respiration, apoptosis, and energy metabolism (level of intracellular ROS, basal respiration, ATP production and nonmitochondrial respiration). Our study improved the human risk assessment of DBPs with the help of a convenient model and parameter and revealed that mitochondrion is a potential toxic focus of DBPs exposure at the cellular level. Moreover, the kidney was supposed to be a target organ of DBPs toxicological effects. Additional researches are needed to determine the effects of DBPs for kidney impairment in other kidney cells (like RPTEC/TERT1 and HK2 cells) and reveal the underlying mechanisms.

Keywords: HEK293 cell; Disinfection byproducts; Toxic potential risk; Kidney

WE016

Effect of PAH mixtures over the glutathione system in HepG2 cells

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Environmental carcinogens affect humans and wildlife by several mechanisms, most of which associated to DNA damage and related pathways. Polycyclic aromatic hydrocarbons (PAHs) constitute one of the major groups of potent carcinogens that are ubiquitous in the environment. PAH require metabolic activation by cytochrome P450 (CYP) mixed function oxidases (MFOs) to elicit their deleterious effects, involving the formation of radical cations, diol-epoxides and redox active *0*-quinones. The reactive metabolites produced during PAH activation may form bulky DNA-adducts, while nucleobase oxidation by action of ROS may also occur. It is well known the link between PAH toxicity and carcinogenicity. However, these studies are based on the results of experimental approaches using exposure to a single compound. Nonetheless, in the environment, PAHs invariably occur in complex mixtures. In fact, previous research, in vivo and in vitro, showed complex interactions when binary mixtures of carcinogenic and non-carcinogenic PAHs were involved. Still, how PAH mixtures modulate toxic pathways remains unknown, especially those which are related to the production of reactive oxygen species (ROS). The aim of the present work was to evaluate the mechanism of action of PAH mixtures on the glutathione system and disclose the interaction effects in human hepatoma HepG2 cells. *In vitro* assays were conducted, exposing HepG2 cells to individual and binary mixtures of two PAHs: phenanthrene (Phe) and benzo[b]fluoranthene (B[b]F), with distinct physicochemical properties and carcinogenic potential. Cell viability was determined by the MTT assay. Total glutathione (GSH) levels and GSH oxidation were evaluated as well as the activities and expression of GSH-dependent enzymes such as glutathione peroxidase (GPx) and glutahione reductase (GR). The production of ROS were also determined. Spectrophotometric and fluorometric techniques were applied. In addition, levels of *S*-glutathionylated proteins (PSSG) and Nrf-2 activation were determined by Western blot and the transcription of target gens was evaliated by qRT-PCR. Results showed a 3-4 fold incrase in GSH levels in mixtures relatively to single PAHs which matches the enahnced Nrf-2 signalling observed. However, PSSG was not increased by mixtures enriched in BbF which could indicate a hampering of GST activity.

WE017

Organohalogenated compounds and heavy metals in farmed and wild milkfish and mullets in Tanzania. Implication for human and fish health risk.

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Fish consumption is known to have several health benefits for humans. However, the bioaccumulation of organohalogenated compounds (organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and perfluoroalkyl substances (PFASs) and heavy metals in fish could pose health risks to human and fish. Therefore in 2016, farmed and wild milkfish and mullet from Tanzania were investigated for these contaminants and assessed for their potential health risk. *p,p'*-DDE and Pb were the major contaminants in the fish. Concentrations of *p,p'*-DDT were 572 times higher in the wild milkfish than in farmed milkfish from the same area. The varying ratios of *p,p'*-DDE/*p,p'*-DDT reflected both historical and recent use DDT in Tanzania. HCB, HCHs and trans-nonachlor concentration were low reflecting positive measures after their ban. Influence of various human activities in Tanzania were reflected by low but varying levels of PCBs and PBDEs. Pb was detected in concentrations above maximum limit (ML) in 100% of the analysed fish from Tanzania. Pb concentrations were higher in wild fish than in farmed fish. This suggest that Pb pollution is more prevalent in wild fish in the Indian ocean than in farmed fish. Whereas the exceeding concentration of Pb might pose potential health risk to human, the concentrations of Hg beyond EQS_{Biota} and high concentration of Pb might affect fish health and can threaten biodiversity and aquaculture development. The estimated weekly intake (EWI), total hazard quotient (THQ) and hazard index (HI) for heavy metals in farmed and wild milkfish and mullet from Tanzania indicated no potential health risk through consumption. However, Pb contributed much to the HI in the fish from Tanzania and it might pose health risk to the communities that mostly depend on fish for diet. In general, this study revealed that wild fish are more contaminated with organonohalogenated compounds and heavy metals than farmed fish. Findings of this study calls for further investigation of sources of Pb, especially in wild fish from the ocean. Moreover, future investigations should include regular monitoring of heavy metals and POPs in farmed and wild fish in Tanzania for further development of aquaculture and the welfare of the wild fish stock in the coastal waters.

WE018
Ecotoxicological assessment of the environmental impact of anti-corrosion coatings
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Steel structures such as bridges, wind turbines and lock gates exposed to the environment are subject to corrosion and need to be protected to improve the durability and thus to ensure their structural stability. Active methods which are using a protective current, and passive methods which mechanically isolate the steel from its environment, can be applied. The latter strategy is commonly pursued by the application of organic coatings which are based e.g. on epoxide and polyurethane resins. In contact with water, various organic substances might be released from the coatings into the environment. Known ingredients of concern for example are Bisphenol A, Nonylphenols and different aromatic hydrocarbons. Furthermore, compounds with unknown ecotoxicological effects might be formed during polymerization (hardening) or the reaction with water. The investigation of possible adverse effects by anti-corrosion coatings on the environment is one aim of a Network of Experts funded by the German Ministry of Transport and Digital Infrastructure (BMVI). For this purpose different coating systems for steel and hydraulic engineering were applied on steel plates and leached with deionized water. To simulate the weathering of coating materials, a subset of plates was exposed to UV-radiation. A range of bioassays was used to investigate the prepared eluates for possible acute toxic impacts on daphnia, freshwater algae and luminescent bacteria as well as mutagenic and estrogen-like effects. Up to now, a legal framework for the assessment of anti-corrosion coatings in Germany is missing. Therefore, an evaluation concept suggested by the German Institute for Building Technology (DIBt) for the assessment of the impact of construction products on soil and groundwater was applied to compare the analyzed coating systems and interpret the toxicological results.

WE019
New natural biocides for application on concrete masonry of historical interest
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In recent years, different research groups have been studying new biocidal products also usable in cultural heritage that are more sustainable for the environment and human health. In particular, these products could be used in restoration procedures to prevent or eliminate biological patinas grown on cultural heritage masonry. Growing interest is now addressed to the use of essential oils (EOs) as antimicrobial substances, in medical, industrial, food, and many other sectors. Oregano and thyme EOs are among the most effective oils and, for this reason, they have also been investigated in the field of cultural heritage. In this research, we developed an OEs-based biocide,applied on a concrete masonry in a monumental complex of historical interest. It had a patina composed of many species of fungi. We developed a blend made of oregano and thyme essential oils, whose active substances are monoterpenes and phenolic monoterpenes. The efficacy was tested by evaluating antimicrobial (and, particularly, antifungal) activity using the Bio-Tape method. The analysis was realised on an area homogeneously covered with biological patinas. The EOs efficacy was evaluated in comparison with 3 commercial biocides: (i) isothiazoline compound (AL); (ii) a combination of isothiazoline and quaternary ammonium salts (BT); (iii) a combination of carbamate and isothiazoline (BR). Our study found that the antimicrobial activity exerted by our OEs blend was comparable to other commercial biocides. Unlike the latter, essential oils have the advantage to be safer and more sustainable than traditional biocides, whose hazard statements and risk phrases have often led to withdrawal from the market of some toxic substances. The future technological development could address the minimisation of the loss of product, due to high volatility of EOs, with formulations which “entrap” the compound, allowing their controlled release to enhance the biocide efficiency.

WE020
Mixture toxicity of copper and its corrosion inhibitor benzotriazole to Daphnia magna
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Benzotriazole (BT) is often used as a corrosion inhibitor in cooling tower pipes, to reduce the dissolution of copper (Cu) in water. Hence, cooling towers regularly discharge wastewater containing a mixture of Cu and BT. Cu and BT are toxic to aquatic biota and affect the health of aquatic ecosystems, but since Cu and BT occur in cooling tower water simultaneously, it is possible that mixture toxicity is even more relevant than their individual toxic effects. Therefore, the aim of this study was to examine mixture toxicity of Cu and BT to the water flea *Daphnia magna*. To this purpose, *D. magna* were exposed to concentration ranges of Cu, BT and Cu+BT mixtures in the 48 h *Daphnia magna* acute immobilisation test in ISO medium. First the toxicity of the individual compounds was determined and the LC_{50} values derived from these experiments were subsequently used to compose equitoxic mixtures of Cu and BT tested in the second experiment. There were five concentrations per compound/mixture, four replicates per concentration and five daphnids per replicate. Dose response relationships were constructed from which the EC_{50}-values were calculated using a non-linear regression model. The EC_{50} value for Cu was 8.9 µg/L (95% confidence interval 6.9-11.0 µg/L) and for BT 147 mg/L (95% confidence interval 126-168 mg/L), comparable to the EC_{50} values obtained from literature. The EC_{50} value of an equitoxic mixture of Cu and BT was 1.16 TU (95% confidence interval 1.01-1.31 TU), which suggests that the combination of both chemicals shows a less than additive toxic effect. An explanation for the less than additive effect of the mixture could be the corrosion inhibiting function of BT. To inhibit corrosion, BT forms a layer on copper pipes and it may also form this layer around copper ions. This way, aquatic organisms could be shielded from the toxic effects of Cu. It is concluded that although a mixture of copper and benzotriazole had a less than additive toxic effect, both chemicals still contributed to mixture toxicity, potentially posing a risk to aquatic ecosystems.

WE021
Mixtures of Antibiotics in wastewater treatment effluents: Is there a risk to the aquatic ecosystem?
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Antibiotics continuously enter the environment because of incomplete removal in wastewater treatment plants (WWTPs). The elimination of antibiotics varies between < 25% and >90% depending on the active substance and technology of WWTPs. In surface water, antibiotics like sulfamethoxazole are frequently monitored and can reach concentrations at the low μg/L range. Among pharmaceuticals, antibiotics are of particular interest, not only because of antimicrobial resistance (AMR) but also due to their potential adverse effects on aquatic organisms. Environmental effectsof human pharmaceuticalsareassessed according to the guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 corr 2). The current regulations take into account only risk assessments for single substances, while effects of mixtures occurring in the effluents of WWTPs are not considered. Therefore, the German Environment Agency (UBA) initiated a project that investigated the effects of several mixtures of antibiotics used in human medicine on cyanobacteria. The toxicity of single substances and mixtures were determined and compared with predicted mixture toxicity. Single-substance and mixture risk assessment were compared in order to explore the relevance of mixtures. Furthermore, the influence of the wastewater effluent matrix on the mixture toxicity was tested. In conclusion, the results demonstrate that ecotoxicological risks of mixtures cannot be excluded in realistic worst-case exposure scenarios for antibiotics.

WE022
Mixtures of Antibiotics in soil: What is known about their occurrence in manure and soil and their adverse effects on soil organisms?
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Antibiotics are largely and increasingly consumed worldwide in human and veterinary medicine. However, they are only partially metabolised and excreted by humans or animals and released in their active form into the environmental compartments such as soil, surface and ground water. This review summarizes the knoweledge on the occurence and effects of antibiotic mixtures in the soil compartment [1]. It is well known that many agricultural soils are contaminated with antibiotics. Even more, it must be expected that agricultural soils not only contain one but mixtures of several compounds. Mixed antibiotic contaminations may result from (i) the administration of combinations of antibiotics, (ii) the medication of different livestock animals and different live stages of the animals with different antibiotics and collection of all excreta in one manure tank and (iii) subsequent, repeated spreading of contaminated manure, sewage sludge or other organic waste materials onto agricultural fields. This results in contaminations of organic waste materials with mixtures of different antibiotics comprising up to 20 or more individual compounds. Consequently, soils fertilized with these substrates contain mixtures of different pharmaceutical antibiotics. This is supported by monitoring data of up to 13 antibiotics found in soil. Existing knowledge on adverse effects of antibiotics on soil organisms is largely restricted to studies with single compounds, which show clear dose-dependent adverse effects for example on microbial biomass, or soil community structure and functions. However, mixtures of antibiotics are of special environmental relevance, since the

ecotoxicity of a pharmaceutical mixture is typically higher than the effects of each individual component. Furthermore, interactions exist with other boundary conditions that affect the fitness of biota such as heat or frost. Despite the outlined findings it must be stated that mostly incomplete knowledge exists on the various topics. More information from targeted, systematic research is needed. To this end, three successive research projects are proposed, aiming to increase the systematic knowledge on effects of mixtures in soil. Not last, all these research efforts should lead to or even be flanked by regulatory measures for an improved use and management of antibiotics. [1] Thiele–Bruhn S. "Environmental risks from mixtures of antibiotic pharmaceuticals in soils – a literature review", UBA Texte (in preparation)

WE023

Environmental risk assessment of emerging organic micropollutants in the Belgian Part of the North Sea

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In the European Union, Environmental Quality Standards for priority substances (and certain other pollutants) are used in regular monitoring to reduce chemical pressure on the aquatic environment. In general, the production and use of most of these priority pollutants are decreasing or have even been banned in Europe. As an example, 15 out of 23 pesticides included in the list of priority substances were banned from the EU market in the past 15 years; leading, however, to the occurrence of other emerging chemicals for replacement. In the present research we performed monitoring of 89 emerging contaminants including pesticides, pharmaceuticals and personal care products and filled ecotoxicity data gaps for approximately 25 of these substances. Effect data was determined using (i) the 72h growth inhibition test with *Phaeodactylum tricornutum* (ISO 10253), and (ii) the 96h acute lethal toxicity test with *Nitocra spinipes* (ISO 14669). This data combined with an extensive literature study was subsequently used to perform an environmental risk assessment for the Belgian Part of the North Sea. The monitoring revealed the presence of 47 organic micropollutants at minimum one of our four sampling locations. While the collected toxicity data covered 56 % of the measured substances with acute effect data for three species (of the three trophic level algae, crustacean and fish), chronic data for species of three trophic levels was available for only 9 % of them. We were able to calculate risk quotients (RQ) for 53 % of the detected micropollutants and found exceedance of the threshold value (RQ > 1) for 5 substances, i.e. flufenacet, metolachlor, metoprolol, pirimicarb and terbuthylazine. The majority (3) of these substances are herbicides which have previously been reported to dominate chemical mixtures and sign responsible for a majority of observable effects in aquatic environments. Here, their exceedance of the threshold was mainly due to low no-observed effect concentrations for algae (metolachlor and terbuthylazine) or crustacean (flufenacet, pirimicarb). While effects of herbicides on algae growth or reproduction are obviously expected, effects on crustacean reproduction are rather not. Overall, our study revealed especially herbicides to potentially threaten Belgian coastal waters. In a refined risk assessment for this ecosystem, we will include potential mixture effects since our monitoring revealed the simultaneous presence of a multitude of chemicals.

WE024

Toxicity of binary mixtures in Danio Rerio: a groundwater case-study

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Groundwater is the primary available source of drinking water across the world, representing one of the most important natural water resources. Due to anthropogenic activities most groundwater aquifers are at risk of chemical pollution. Groundwater aquifers are also essential for agricultural irrigation and for sustaining natural ecosystems functioning and health. The Groundwater Directive (2006/118/EC) was created to protect groundwater bodies from chemical pollution by setting quality standards and establishing threshold limits for a set of priority pollutants and by this way avoid deterioration in the quality of such water bodies. These type of regulations are applied based on a one-by-one chemical assessment, disregarding any interaction between them. Therefore, it is crucial to conduct research to identify possible contaminants that may pose a risk to groundwater ecosystems and help improve quality criteria. The present work was performed in the context of the European Research Project WE-NEED (Water JPI- WATERWORKS2014 ERA-NET) focused on developing new management strategies to sustainably exploit groundwater resources. Acetaminophen, triclosan and PFOS (Perfluorooctane Sulfonate) are contaminants commonly found in groundwater aquifers across Europe and therefore were selected for this case-study using the Cremona aquifer in Italy. Synthetic water was built to mimic the aquifer's groundwater composition and acute single and binary mixture Fish Embryo Toxicity Tests (FET) were performed with *Danio rerio* (adapted from OECD 236; Lammer et al. 2009) to predict their mixture toxicity. Single exposures revealed Triclosan as the most toxic chemical tested to *Danio rerio*. The reference model of Concentration Addition was applied to analyse the binary mixture results and deviations from additivity were found, which indicated interaction between the tested chemicals.

WE025

Derivation of total polycyclic aromatic hydrocarbon (PAH) water quality standards using probabilistic approaches

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PAHs are ubiquitous environmental pollutants arising from industrial activity involving oil and gas. They have been shown to be toxic, mutagenic and/or carcinogenic to the environment and human health and are thus targets for regulatory management. A number of jurisdictions have derived environmental quality standards (EQSs) for PAHs that cover only a few individual compounds (e.g. naphthalene, fluoranthene). Alternatively, some jurisdictions have set a "PAH group" quality standard, by selection of a number of PAHs for analysis (e.g. denoted as PAH-6 or PAH-16). In this study we attempted a comprehensive compilation and assessment of the available long-term ecotoxicity data for PAHs and assessed the possibility to update EQSs for individual PAHs. Ecotoxicity data underpinning long-tem PAH standards developed by various jurisdictions were reviewed, forming a foundation dataset. Further searches of the published literature were conducted to identify additional long-term hazard data relevant to the derivation of water quality standard values. The acceptability of the data were categorised as primary, secondary or unacceptable, according to the Canadian Council of Ministers of the Environment (CCME) aquatic protocol (2007). This exercise highlighted that, with a few exceptions, the available dataset of long-term ecotoxicity data is insufficient to derive long-term water quality standards for freshwaters, marine waters or sediments for individual PAH compounds. This appeared to be the case regardless of the jurisdictional approach to water quality standard employed (e.g. Europe, Canada, USA, Australia and New Zealand). Since any number of PAHs from various sources will be present in the environment, with each location having a unique PAH composition, consideration of the entire PAH dataset as a whole may be useful in addressing the data paucity for individual substances. A "total PAH" standard, considering relevant data from all PAHs, may thus be a simpler approach to monitoring PAH contamination of the aquatic environment. Primary and secondary ecotoxicity data for individual PAHs were aggregated to calculate species sensitivity distribution (SSDs) for long-term freshwater, marine water and sediment. Total PAH standards were then derived based on the SSDs, taking account of the approaches recommended for standard derivation in Europe, Canada and Australia. We compared the proposed total PAH standards with existing standards for individual PAHs and PAH groups.

WE026

The ecotoxicity profile of deep eutectic solvents through the eyes of the mixture toxicity theory

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The interest on DES has been increasing, boosted by their advantages over conventional solvents. However, the ecotoxicological profile of DES, particularly non cholinium-based DES, is scarcely known. Also, despite previous studies showed that DES components dissociate in water, no previous study assessed DES toxicity using the classical models for mixture effect prediction - concentration addition (CA) and independent action (IA). In this study we evaluated the ecotoxicological profile of DES based on the quaternary-ammonium salts tetramethylammonium chloride ($[N_{1111}]Cl$), tetraethylammonium chloride ($[N_{2222}]Cl$) and tetrapropylammonium chloride ($[N_{3333}]Cl$), (as hydrogen bond acceptors, HBA) and ethylene glycol and 1-propanol (as hydrogen-bond donors, HBD). In particular, this study aims to assess the ecotoxicological profile of these DES with *Allivibrio fischeri*, using the Microtox® Acute Toxicity Test. The mixture models CA and IA were used to analyse DES toxicity allowing the identification of synergistic/antagonistic, dose-ratio and dose-level effects between the HBA and HBD components of the DES. These deviations to the baseline models allow a greater detail in understanding the relation between HBA and HBD. DES toxicity followed the same trend as observed for the salts: $[N_{1111}]Cl$-based DES < $[N_{2222}]Cl$-based DES < $[N_{3333}]Cl$-based DES, being 1-propanol:$[N_{3333}]Cl$ the most toxic (30 min-EC_{50} between 1.120 and 4.981 g/L). The IA model, with specific deviations, adjusted better in 5 out of 6 DES. Antagonism was observed for $[N_{1111}]Cl$-based DES, and synergism for $[N_{3333}]Cl$-based DES and for 1-propanol:$[N_{2222}]Cl$. As demonstrated for the first time in this study, the application of the mixture toxicity models represents a breakthrough in the problematic of assessing DES toxicity, given the potentially countless number of DES that can be created with the same starting materials. The graphical representation of the response curves will allow the prediction of DES toxicity (varying ratios of the same HBA:HBD) without having to test them all experimentally. CICECO and CESAM were financed by national funds through the FCT/MEC and when co-financed by FEDER under the PT2020 Partnership Agreement (UID/CTM/50011/2013 and UID/AMB/50017/2013). FCT funded IPE Macário, AMM Gonçalves, JL Pereira and SPM Ventura (SFRH/BD/123850/2016, SFRH/BPD/97210/2013, SFRH/BPD/101971/2014 and IF/00402/2015), and supported the research through the project Ref. PTDC/ATP-EAM/5331/2014.

WE027

Toxicity of mixtures of ionic liquids and salts to the standard freshwater microalga Raphidocelis subcapitata

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Ionic liquids (ILs) are salts at the liquid state at temperatures below 100 ºC. These are often described as “green solvents”, given their unique properties, such as negligible vapor pressure, good solubility for many organic and inorganic materials, and theoretically low toxicity. ILs have several industrial applications, being used solely or together with other compounds, namely salts. If mixtures of ILs and salts reach the aquatic systems, they can cause adverse effects to aquatic biota. Despite previous studies have addressed the toxicity of IL’s to aquatic organisms, the mixtures of IL’s with salts have been overlooked. The main goal of this work was to assess the toxicity of binary mixtures of ILs (cholinium bitartrate, cholinium dihydrogencitrate, cholinium bicarbonate and benzyl dimethyl ammonium chloride) and salts (potassium phospate and sodium citrate) to the microalga *Raphidocelis subcapitata*. The freshwater microalga was exposed to these chemicals individually and in mixture, during 96h, and the yield was measured to calculate EC_{50} values. The toxicity of the ILs varied by a factor of 10, highlighting the role of the anion in the toxicity of the IL. Their toxicity increased in the following order: benzyl dimethyl ammonium chloride (EC_{50}=1060.3 mg/L) < cholinium bicarbonate (EC_{50}=649.2 mg/L) < cholinium bitartrate (EC_{50}=138.2 mg/L) < cholinium dihydrogencitrate (EC_{50}=100.8 mg/L). These values agree with the benign nature of the tested ILs. Concerning salts, potassium phospate was less toxic (EC_{50}=598.0 mg/L) than sodium citrate (EC_{50}=393.9 mg/L). Regarding the effects of the mixtures, both synergism and antagonism deviations were found, however antagonism was the most common.This work raises concern about the toxic effects of mixtures of ILs and salts towards microalgae, especially for combinations where synergistic effects were found. Results suggest that the toxicity of ILs might be under- or overestimated when they are tested individually. Ultimately, this work highlights the importance of testing the toxicity of mixtures given their ecological relevance in aquatic systems. CICECO and CESAM were financed by national funds through the FCT/MEC and co-financed by FEDER under the PT2020 Partnership Agreement (UID/CTM/50011/2013 and UID/AMB/50017/2013). FCT funded AMM Gonçalves, JL Pereira and SPM Ventura (SFRH/BPD/97210/2013, SFRH/BPD/101971/2014, IF/00402/2015), and supported the research through the project Ref. PTDC/ATP-EAM/5331/2014.

WE028

Environmental Risk Assessment of Illicit Drugs in effluents from WWTPs and in the recipients

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The presence of illicit drugs in effluents from Wastewater Treatment Plants (WWTPs) and their recipients has raised concern over their possible negative effects on aquatic ecosystems. The purpose of this study was therefore to determine the ecological risk of five illicit drugs Cocaine (COC) and its metabolite Benzoylecgonyne (BE), MDMA, Amphetamine (AMPH), Methamphetamine (METH), and Cannabis (THC/THCA) in effluents and recipients in the Halland County, Sweden. The concentrations of illicit drugs were measured and calculated in accordance with the guidelines for the EMCDDA, 2017 on WWTP’s effluents from 10 WWTPs sampled in September and November, 2017 by the Academic Research Center for Chemicals, Health and Environment (SWETOX). The measured values were used as PEC values. Predicted No Effect Concentration (PNEC) values (acute and chronic) were estimated based upon ecotoxicological data found in the literature and by using ECOSAR software (version 2.0). Method of selecting Assessment Factors (AF) were based upon the legislation for chemicals in EU. An approach suggested by Backhaus and Faust (2012) was used to calculate the risk quotients (RQ) of the mixture of the illicit drugs (MRQ). A dilution factor of 10 was used to estimate RQs of the recipient surface waters. RQs higher than 1 (RQ>1), were considered as a potential of risk on the aquatic environment. $RQ_{chronic}$ values for the illicit drugs in the effluents varied between 0 (concentration below detection limit) and 2.6. $MRQ_{chronic}$values in the effluents varied between 0.003 and 2.7. Effluents from three WWTP showed RQ>1 and MRQ> 1 and the presence of THCA in elevated concentrations mainly contributed to the result. Using a default dilution factor of 10 for potential risk in surface water resulted in RQ and MRQ values Reference: Backhaus T and Faust M. (2012). Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environmental Science Technology 46:2564-2573.

WE029

Extreme flooding events impact groundwater quality following natural disasters in Texas, USA

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Hurricane Harvey dropped 102-155 cm of rain totaling 76 trillion liters of water on the central and upper Gulf Coast areas of Texas in 2017 causing widespread flooding, including flooding of private water wells, oil refineries, industrial facilities and at least 13 Superfund sites in the Houston metroplex, home to about 7 million people. Following the hurricane, collaborators from Virginia Polytechnic Institute and State University, Louisiana State University and the Texas A&M AgriLife Extension Service conducted private water well sampling events. Well water samples were analyzed for indicator bacteria (total coliform and *Escherichia coli*), inorganics including trace elements and heavy metals, and waterborne pathogens including *Legionella* spp., *Naegleria fowleri, Giardia lamblia*, shiga toxin-producing *E. coli*, *Vibrio vulnificus,* and *Mycobacterium* spp. (pathogen results to be reported elsewhere). More than 1,500 private water wells were tested through 60 events serving 29 hurricane-impacted counties, allowing for examination of spatial and temporal effects on concentrations. Sample sizes varied for particular analytes. In addition to spatial-temporal effects on contaminant concentrations, results also will be coupled with private well participant survey data including 1) resident demographics; 2) extent of flooding and well damage, 3) well construction and design parameters; 4) well water use and consumption patterns, and use of water treatment; and 5) activities, attitudes, obstacles, and motivations related to well testing, maintenance and disinfection, flood preparation and recovery, and service provision. Another extreme flooding event occurred in the central Texas hill country in 2018. As during the flooding that occurred with Hurricane Harvey, survey results paired with analyses for *E. coli* indicated that 24% of water wells that well owners reported were submerged contained *E. coli* while only 3% of wells that were not submerged contained *E. coli*.

WE030

Towards Increased Realism in the Site-Specific Risk Assessment of UVCBs - Leveraging On-site Monitoring Data and Industrial Hygiene Modeling Approaches in Environmental Risk Assessment

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Significant work has been done over the past several decades to accurately define and predict the processes that control the environmental emissions, transport, and ultimate fate of chemicals during the manufacturing and formulation stages of a chemical’s life-cycle. Much of the improvements to chemical emissions during manufacture and formulation have come from the development of sector- and use-specific specific environmental release conditions (SpERCs). Despite significant improvements to these release scenarios, there has largely been a disconnect between environmental and worker (human health) exposure and risk assessments, in many cases leading to unreasonably high emissions factors (EFs) in environmental risk assessments, which are incongruent with both occupational exposure limits (OELs, TLVs, etc…) and on-site monitoring results for industrial sites. These problems are compounded by the complexity of UVCB substances and the presence of mixed waste streams (i.e., TPH) and non-targeted monitoring data (i.e., total VOC). The purpose of this work is two-fold – (1) to provide a consistent, reproducible approach for incorporating on-site monitoring data (air & water) to support significant reductions in environmental RCRs versus a generic exposure assessment using default emission factors (SpERCs); and (2) to provide a scientific basis for the use of industrial hygiene and worker exposure criteria and monitoring results to “ground-truth” and refine environmental emission factors.

For several UVCB petroleum streams generic exposure assessments are compared to site-specific assessments using on-site monitoring data using the PetroRisk 7.07 environmental risk assessment tool.

WE031

Fish primary hepatocytes as a tool to assess the toxicity of complex mixtures of Polycyclic Aromatic Hydrocarbons.

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Monitoring carcinogenic substances has long been considered a priority in risk assessment strategy worldwide, whether it concerns human or ecological health. Polycyclic aromatic hydrocarbons (PAHs) are classified as priority substances and are ubiquitous environmental carcinogens that occur in complex mixtures. In spite much research, environmental guidelines for PAHs are invariably drawn for single substances which compromises risk assessment. In fact, previous research with seabass revealed that the interactions between PAHs of distinct classes (carcinogenic and non-carcinogenic) may generate effects more complex than could be anticipated, by mechanisms not yet fully understood. The toxicity of PAHs is derived by their highly reactive metabolites produced by CYP monoxygenases. Simultaneously, reactive oxygen species (ROS) are also produced as by-products. These reaction products may cause DNA damage hence their recognised genotoxic, mutagenic and carcinogenic potential. Moreover, PAHs may mediate the induction of CYP enzymes through the aryl hydrocarbon receptor (AhR) pathway. However, the toxicological mechanisms of complex mixtures of PAH remains largely unknown. In vitro assays with stable cell lines are being regarded as a convenient approach to meet the 3-Rs policies (reduce, refine, replace) regarding experimenting with laboratory animals. The use of primary cell cultures from wild organisms has been gaining relevance in ecotoxicology as they are generally considered to be more sensitive than stable cell lines and retain most of their original biochemical pathways. The present research evaluates the toxicological effects of complex mixtures of PAHs using in vitro tests with primary hepatocytes from an ecologically and economically-relevant marine fish. The endpoints measured include viability assessment by the MTT assay and LDH leakage, evaluation of phase I metabolism (e.g. CYP1A1 activity and expression) and Phase II enzymes of PAH metabolism, such GST activity. Integration of results seeks to validate this in vitro approach as an efficient tool to disclose the interaction effects of distinct PAH mixtures.

WE032

Examining the interrelationships between chemical/non-chemical stressors and inherent characteristics in children with ADHD

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Children may be more vulnerable to the combined interactions of chemical and non-chemical stressors from their built, natural, and social environments when compared to adults. Up to 20% of children are diagnosed with a mental illness annually in the United States with a large number not receiving adequate treatment. The Centers for Disease Control and Prevention estimates that 11% of children have ADHD. ADHD is characterized by developmentally inappropriate levels of hyperactivity, impulsivity, and inattention, with the neurotransmitter serotonin regulating these symptoms. Monoamine oxidase A (MAOA) aids in serotonin uptake and has two genotypes that affect its enzymatic activity (low: L; high: H). MAOA-L carriers are more frequently diagnosed with behavior and emotional disorders, including ADHD. When children are exposed to cigarette smoke, bisphenol A (BPA), or organophosphate pesticides, MAOA activity is inhibited. Non-chemical stressors, such as traumatic childhood experiences, ethnicity, and lifestyle factors, complicate the relationship between genotype and chemical exposures. But, the co-occurrence among outcomes between chemical exposures, genotype, and non-chemical stressors with MAOA-L suggest that mental illness in children may be caused by multiple interacting factors. This project aims to elucidate the links between chemical and non-chemical stressors and inherent characteristics on children's mental health outcomes using a systematic review and meta-analysis to align results within our multifactorial conceptual framework. Preliminary results show that prenatal and childhood exposures to recreational drugs, lead, organic contaminants, and phthalates are associated with greater odds of being diagnosed with ADHD. MAOA genotype and early childhood experiences also influence mental health outcomes associated with ADHD. An improved understanding of inherent vulnerability and the onset of mental illness may provide better intervention and treatment options to children in need.

Statistical Science and Ecotoxicology: Bright Lines and Dark Alley Ways (P)

WE033

When p is not of value anymore - effect sizes and their uncertainties as an alternative to interpret ecotoxicological data

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Null-hypothesis significance testing (NHST) is still a commonly applied statistical tool in ecotoxicology despite decades of controversy about its focus on the *p*-value irrespective of the biological relevance of effects. However, for many questions in science and management, it is generally more valuable to know the probability of a specific hypothesis or effect size, rather than a statement about whether data differ significantly from those expected under the null hypothesis. Thus, we suggest results should emphasize effect sizes and the degree of uncertainty surrounding their estimations in a frequentist or Bayesian framework. To examine this, we compared how NHST and effect size-based statistics can affect inference from ecotoxicological datasets by applying (i) analysis of variance models using NHST and (ii) effect sizes using both frequentist confidence intervals and Bayesian frameworks. In many cases, the results were similar among the three approaches: NHST *p*-values were < 0.05 when effect sizes were $\geq$10% and Bayesian probabilities exceeded 80%, thus indicating biologically relevant effects. In several cases, however, NHST-results provided different interpretations than those revealed by effect sizes and respective Bayesian probabilities: biologically relevant effects were not revealed by NHST (p>0.05) despite effect sizes in the range of 10-40% and Bayesian probabilities of 80-100%. Furthermore, NHST drew attention to a biologically irrelevant effect, which happened to be statistically significant (p=0.049; effect size: 5%; Bayesian probability: 72%). Effect sizes and respective uncertainties as well as Bayesian probabilities provide a more nuanced interpretation of results, as these methods allow scientists to form conclusions regarding biological relevance based on their expert judgement and effect thresholds. We conclude that ecotoxicology should ultimately move from conventionally applied NHST that relies on *p*-values towards an effect size-based approach to maximize the probability of identifying biologically relevant effects.

WE034

How to prove there is no effect: The MDD concept and alternative indicators for the interpretation of ecotoxicological study results

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Risk assessment of pesticides has to evaluate potential effects on non-target organisms. In addition to laboratory tests, mesocosm and field studies are regularly conducted to show the absence of such effects, often with small sample sizes. When statistical non-significant results are reported by such a study, it is often challenging to distinguish between the true absence of an effect (true no-effect), and lack of power, meaning that the study failed to detect an existing effect (type II error). Methods for judging whether a non-significant result does in fact indicate the absence of an effect include confidence intervals (CIs), post-hoc power analysis and the post-hoc calculation of a minimum detectable difference (MDD). Although limitations of post-hoc power analysis and MDD have been acknowledged widely (including EU and US regulatory agencies), the MDD is still recommended in current guidelines for ecotoxicological risk assessment. Moreover, the MDD concept, traditionally mainly applied to simple experimental contrasts, has recently expanded in use also to LMMs and GLMMs, without solid evidence of its validity in this context. We use simulations and real-world case studies to investigate the ability of CIs, post-hoc power analysis and MDD to distinguish between true no-effects and type II error. We also investigate whether a direct relationship between MDDs and CIs exists in general, in particular for more complex (generalized linear) models, as suggested in the literature. We compare the different concepts regarding their ability to create correct decisions in a regulatory context and give recommendations for revised statistical protocols to show the absence of an effect.

WE035

Validation of augmented species sensitivity distributions for fish - a new non-testing approach to refine acute risk assessments of fungicides

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The aquatic risk assessment of plant protection products (PPPs) in the EU follows a tiered approach. The standard acute risk assessment for fish (tier 1) is based on the rainbow trout 96-h LC_{50} value divided by an assessment factor of 100. If unacceptable risk is indicated at tier 1, a species sensitivity distribution (SSD) approach is one method to refine the acute risk to fish. SSDs are cumulative distribution functions of toxicity values of multiple species, which are used to estimate the hazardous concentration potentially affecting 5% of the species (HC_5). According to the EFSA Aquatic Guidance Document at least 5 toxicity values are required for a fish SSD. However, this additional acute fish testing conflicts with the wish to minimise vertebrate testing as much as possible for animal welfare reasons. The most effective method to solve this dilemma is by leveraging the existing data. Following this idea, experimental fish toxicity data for standard species were augmented with toxicity values calculated with interspecies correlation estimation (ICE) models to construct SSDs. ICE models predict the acute toxicity of untested taxa from experimental toxicity data of a tested species. HC_5 values derived from fish SSDs augmented with ICE-derived toxicity values (augmented SSDs) were validated by comparison with HC_5 values from SSDs based on experimental data (reference SSDs). This exercise was done for fungicides covering different modes of action, because fish frequently drive the aquatic risk assessment of fungicides leading to the need for refinements. Our work shows that this method provides robust SSDs for use in risk assessment without the need for additional fish testing.

WE036

Improved Experimental Design for Non-Target Terrestrial Plant Studies

J.W. Green, JohnWGreen-ecostats.com / Data Science and Informatics

Regulatory guideline studies for Non-Target Terrestrial Plant (NTTP) are Seedling Emergence (OECD TG 208) and Vegetative Vigor (OECD TG 227). Both have growth responses (dry or wet weight and shoot height) and survival (TG 227) or emergence (TG 208). An extensive database was explored to find what experimental designs allowed adequate ability to estimate ECx, x= 10 to 50, and determine a NOAER. Design considerations were the number of application rates, replicate test vessels (pots) per application rate, plants per rep, and whether to have the same number of reps at all rates or to increase the number of control reps (e.g., using a square-root allocation rule). The decision criteria were the power of multiple comparison methods to establish a NOAER and the distribution of ECx estimates and their confidence bounds. The database determined the distribution of the within- and between-rep variances (VARERR and VAREEP, respectively) and the ratio VARREP/VARERR, as well as the range of rate-response shapes in such studies. A realistic simulation study was then developed to explore alternative designs. For emergence studies, a design with 6 application rates (including a zero-rate control), 8 reps/rate, 5 plants/rep, with equal allocation of reps to rates, provides NOAER determination and EC10 and EC25 of comparable power and quality to those possible from larger designs. For Vegetative Vigor studies, a design with 8 rates with 5 reps of 4 plants each, with equal allocation of reps to rates allows NOEC determination and EC10, EC25, and EC50 estimation with comparable power or quality to that possible under larger standard experiments. These conclusions must be qualified: Shallow rate-response curves occur frequently and vary across species and chemicals in mostly unpredictable ways. Both NOAER and ECx estimates suffer for such data. Quality of results depends on the statistical models and model selection criteria, and multiple comparison tests. Specific recommendations are provided.

WE037

Model Selection Criteria for Growth Responses in Non-Target Terrestrial Plant Studies

J.W. Green, JohnWGreen-ecostats.com / Data Science and Informatics

Growth responses in NTTP studies are dry or wet weight and shoot height. There is no *apriori* model for such data and for a single dataset, ERx estimates can vary substantially across different models that are plausible, at least in the abstract. It is thus necessary to define objective goodness-of-fit criteria and model selection criteria to select which, if any, model from an appropriate suite of models, provides reliable estimates of ERx and for what values of x. The suite of models explored are Bruce-Versteeg "probit type," 3-parameter log-logistic, Brain-Cousens hermetic, and 2- and 3-parameter exponential models with and without an additional parameter defining a minimum level possible or "floor." Including a floor is sometimes useful avoid estimating responses below any biologically plausible level (e.g., a plant will die before it shrinks to 1% of normal weight). Goodness-of-fit criteria include the following. ERx is estimated as a change from the model estimated control mean, so model agreement with the observed control mean is important. Somewhat less important is agreement of the model with observed treatment means, especially near the estimated ERx rate. If notable low dose stimulation is observed, the model should reflect it, since otherwise, the estimated control mean tends to be pushed towards the stimulated value. The width of the confidence intervals for ERx and the predicted response at ERx should not be overly wide. The model should not be sensitive to one or two observations and all model parameters should be significantly different from zero. Model selection criteria include Akaiki's AICs or BIC and an assessment of how well models being compared meet the goodness-of-fit criteria. Results from a large NTTP database indicate the relative success of these models in fitting NTTP growth data using the stated criteria and the size effects that can be estimated from such studies under current test guidelines.

WE038

Causal inference in ecological risk assessment of heavy metals for benthic invertebrate community in rivers

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In ecological risk assessment for heavy metals, field surveys in rivers provide valuable information about the community-level responses of aquatic organisms to heavy metal pollution. Field survey-based datasets have frequently been analyzed based on correlation analysis such as a method of quantile regression analysis. Correlation analysis neglects the effect of confounding factors that affect both a biological indicator and its limiting factors. Confounding factors may cause spurious associations, and the misestimation of ecological risk derived spurious associations from leads to inappropriate management action. Here, we demonstrated the importance of causal inference in ecological risk assessment of heavy metals using a field survey-based dataset targeted to heavy metals and benthic invertebrates in 19 rivers in Japan. We developed the causal diagram comprised of the biological indicator (Ephemeroptera, Plecoptera, and Trichoptera [EPT] richness), quality of river water (e.g., pH), and physical characteristics of river (flow velocity of river and riverbed structure). Based on the diagram, we constructed a multiple regression model with covariates satisfying the back-door criterion for estimating the causal effect of heavy metals. We did not confirm the causal effects of heavy metal concentrations on EPT richness in the multiple regression model. There was a significant causal effect of flow velocity on EPT richness. The concentrations of heavy metals were correlated to flow velocity in our dataset; therefore, a spurious negative association between EPT richness and heavy metal concentrations was attributed to the correlation. These results suggested that flow velocity needed to be regulated to examine the causal effects of heavy metals on EPT richness in the field. When field survey-based dataset is comprised of several rivers, EPT richness, heavy metal concentrations and flow velocity usually differ in each river. Therefore, all these variables in our dataset were affected by the variable of "river" which is the confounding factor. Consequently, misestimation of ecological risk assessment of heavy metals occurred when assessment was based only on correlation analysis.

WE039

Improving the reliability of ecotoxicological impact assessment metrics - A combination of Quantitative Structure-Activity Relationships and Interspecies Correlation Estimation equations

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The Hazardous Concentration (HC50) is used as a measure of the potential effect of a chemical on an ecosystem. HC50s describe the concentration of a chemical in the environment above which 50% of the species in a given ecosystem are above their EC50, meaning the effective concentration at which 50% of a population displays an adverse effect. EC50s are either experimentally derived or estimated with Quantitative Structure-Activity Relationships (QSARs), both implying a certain level of uncertainty thus influencing the uncertainty of the HC50. The uncertainty of the HC50 is further also driven by the number of species for which EC50s are available: the lower the number, the higher the uncertainty. The resulting uncertainty ranges of HC50s make the interpretation of ecotoxicological assessments challenging. We therefore propose an approach to increase the reliability of HC50s by combining EC50s estimated with QSARs and Interspecies Correlation Estimation (ICEs) equations. ICEs estimate the ecotoxicity of a chemical to a species from the known toxicity of the chemical to another species. This approach was applied to a large set of chemicals focusing on the ecotoxicity for freshwater species. In total, four sets of HC50s were compared: HC50s derived from (1) three species-specific EC50s estimated from QSARs, (2) three species-specific EC50s from QSARs and the 97 available ICEs, (3) three species-specific EC50s from QSARs and per species the least uncertain ICE, and (4) experimental EC50 values for four to six different species. The uncertainty of the HC50s was quantified with a Monte Carlo analysis (10'000 iterations) propagating the uncertainty of the EC50s derived from experiments, QSARs, and ICEs as well as the sampling uncertainty. We used the uncertainty ratio (UR = 95^{th} percentile over 5^{th} percentile) to measure the change in uncertainty for each set of HC50s. Using four commonly used solvents as case studies, we showed that for the HC50s derived from estimated EC50s increasing the number of species from three to six reduced the average UR from 7.8E7 to 6.6E3. When all ICE equations were used the UR dropped to 5.6E1. This was higher than the UR of experimentally derived HC50s (1.5E1). We showed an increased reliability of HC50 values derived from more EC50s despite the use of estimation techniques implying a certain uncertainty level. Future work will present results for a wider set of chemicals and other ecotoxicological impact assessment metrics.

WE040

MOSAIC: a web interface for statistical analyses in ecotoxicology

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University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology

In ecotoxicology, toxicity tests are standardly conducted in order to measure acute or chronic effects of potentially toxic substances on reproduction, growth and/or survival of living animals. Based on statistical models, mainly exposure-response/effect models, these data are analyzed to derive toxicity thresholds associated with their uncertainty, thus helping risk assessors in taking argued decisions. But choosing the most appropriate model and tracking uncertainties is not always an easy task if not implemented in ready-to-use software. MOSAIC, standing for MOdeling and StAtistical tools for ecotoxICology, is developed since 2013 to meet this user request for a freely available user-friendly web interface dedicated to the mathematical and statistical modelling of standard toxicity test data. MOSAIC is available at http://pbil.univ-lyon1.fr/software/mosaic/. Its simple use makes MOSAIC a turnkey decision-making tool for ecotoxicologists, regulators and risk assessors. Indeed, without requiring immersing in extensive mathematical and statistical technicalities, users are provided with advanced and innovative methods to get quantitatively robust estimates of toxicity thresholds in support of their daily work in environmental risk assessment (ERA). Most of MOSAIC functionalities are based on Bayesian inference. From the joint posterior distribution of parameters, Bayesian inference allows a clear handling of uncertainties, that is propagated to the derivation of the toxicity thresholds. In this poster, we first illustrate how to derive LCx and ECx estimates in few steps from standard toxicity data. Then we illustrate how to perform an SSD analysis including censored data, that is data defined as intervals (e.g., an LC50 value known to be smaller than an upper bound, greater than a lower bound or between two uncertainty limits). At last, we present the most recent tool available in MOSAIC, which is dedicated to the use of toxicokinetic-toxicodynamic (TKTD) models, especially General Unified Threshold models of Survival (GUTS models), to fit survival data as a function of both time and concentration. This new functionality could become the new paradigm to derive precise LCx estimates with a better precision than the ones obtained from classical exposure-response/effect models.

WE041

MORSE: An R-package dedicated to ecotoxicology

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The effects of chemicals on living animals are usually measured on individual life history traits according to standardized toxicity laboratory tests. This ensures the control of experimental conditions and the reproducibility of the toxicity tests which generally concern survival, reproduction and/or growth of animals. The statistical analysis of the corresponding collected data leads to the estimation of critical effect concentrations. If toxicity tests have been carried out with a reasonable number of concentrations of the studied chemical substance and if the responses or the effects are statistically significant, a regression model is recommended in order to estimate LCx and/or ECx at the end of the experiment. However, choosing the most appropriate exposure-response/effect model may be not trivial while it may have a strong impact on the resulting threshold estimates. In addition, these models prevent the full exploitation of available data, monitored over time, thus leading to a statistical bias in time-dependent toxicity threshold estimates. They are also only defined for constant exposure concentrations, making not possible the extrapolation of the results to more realistic, time-variable exposure profiles. Fortunately, such situations can be accounted for with the use of toxicokinetic-toxicodynamic models, as for example the General Unified Threshold model of Survival for survival data. Nevertheless, proper and relevant estimates of parameters for all these models are rarely possible within a unique software, nor barely feasible for non-statisticians. To bridge this gap, we developed ready-to-use functions within an R-package entitled 'morse' (standing for MOdelling tools for Reproduction and Survival data in Ecotoxicology), freely available at https://CRAN.R-project.org/package=morse. The R-package 'morse' is based on a Bayesian framework to provide parameter estimates as probability distributions, from which uncertainties can be propagated to any output of interest. This poster illustrates: (1) how simple it is to fit an exposure-response/effect or a GUTS model with only few lines of R code; (2) how to estimate LCx either from a classical exposure-response/effect or a GUTS model; (3) how to make predictions of the survival rate under untested time-variable concentration profiles; and (4) how to get an estimate of the new concept of x% lethal profile (LPx) as promoted by the recent Scientific Opinion from EFSA.

WE042

GUTS Shiny App: a web platform to simulate survival rate under time-variable exposure concentration profiles.

A. SIBERCHICOT, University Lyon 1; V. Baudrot, CNRS / BioSP; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology

Environmental Risk Assessment is based on fitting standard dose-response models to toxicity test data, and LC/EC50 are estimated at the end of the experiment. This common practice prevents the full exploitation of available data, which are monitored over time, leading to a statistical bias in the estimation of time-dependent toxicity thresholds. In addition, standard models are only defined for constant exposure concentrations, making impossible the extrapolation of the results to more realistic, time-variable exposure profiles. Toxicokinetic-toxicodynamic (TKTD) models may help in bridging this gap, as they describe chemical effects by considering the exposure dynamics. The most significant advantage of TKTD models is the possibility to calculate LC/ECx for any exposure duration t, even at time points outside the experimental design. TKTD models also support a mechanistic understanding of the chemical mode of action, the derivation of time-independent effect parameters and the prediction of risks under time-variable exposure profiles. Despite these manifest advantages, TKTD models are not widely used in practice, due to their mathematical complexity that requires advanced methods such as numerical integration and non-linear optimization to fit them to experimental data. In its recent Scientific Opinion, among TKTD models, EFSA promotes the use of General Unified Threshold models of Survival (GUTS) for regulatory risk assessment of pesticides for aquatic organisms. GUTS models are fitted on experimental data to estimate parameters, the survival rate is predicted under environmental exposure scenarios, and predicted numbers of survivors are compared to observed data. Predictions are then validated based on three criteria: the % of data predicted within uncertainty limits of predictions, the normalized root mean square error and the final survival probability prediction error. This poster presents the GUTS Shiny App (http://lbbe-shiny.univ-lyon1.fr/guts-shinyapp/), a new web platform to simulate GUTS models online for any time-variable exposure concentration profile. If uncertainty on input parameters is available, it is propagated to the model outputs. Following EFSA recommendations, the GUTS Shiny App allows the estimation of the multiplication factor causing an additional x% of mortality at the end of the profile (the so-called x% lethal profile or LPx). If users download observed data, the GUTS Shiny App automatically delivers EFSA validation criteria.

Assessment of Social Impacts for Decision-Making Processes and Communication (P)

WE043

Investigation of medicines consumption and disposal by the Brazilian population

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The environmental concern regarding pharmaceuticals pollution has been increasing. There are many pathways of pharmaceuticals to reach the environment, in which the incorrect disposal of medicines by the population is one of those, and many countries still lack effective regulation. When pharmaceuticals reach the ecosystems, they may cause adverse effects on organisms. Therefore, generating information about the disposal of medicines by the population is essential as well as bring knowledge to society. Then, the objective of this research is to investigate the consumption habits and disposal of medicines among the Brazilian population. This study also aims to bring knowledge to the community, as well as generate awareness about the use and disposal of drugs by the Brazilian population. For this, questionnaires were applied by an online platform. In total, 540 forms were answered. Within the participants taking medicines currently (57.4%), the majority takes one to two medicines per day (41.7%). 64% of the respondents have the habit to self-medicate, and 40.2% have already felt some side effects. Analgesics were the class of medicines more mentioned by the participants. 60.4% of the respondents discard their disused or expired drugs in common garbage, and 54.4% of respondents do not know if their cities have specific collectors of medicines. A large respondent percentage (71.9%) never receive any information about correct disposal of medicines, and 95.2% of the respondents believe that pharmaceuticals residues can be harmful to the environment. Many conferences have already mentioned the need for environmental education since it is the base to environmental health and management. Therefore, efforts to raise environmental awareness will beneficially affect drug disposal practices. Although many countries still do not have adequate protocols for unused medicines disposal, it is essential that the population is aware of the incorrect forms and consequences for the environment. This study emphasizes the need for greater incentive in environmental education and public policies related to the pharmaceuticals use and disposal. Furthermore, we believe that the conscious consumption of medicines, which means in the correct accomplishment of medical treatments, reduction of self-medication, and proper destination of these compounds are significant for the mitigation of environmental impacts caused by pharmaceutical pollution.

WE044

Sustainable Sediment Management: Whose Values Are We Sustaining?

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Sediment management is not a sustainable practice. We manage sediment to support specific goals or to address past, unsustainable practices. All active management results in (desirable and undesirable) environmental, economic and social impacts. Given the uncertainty inherent in many management activities, sometimes we are addressing how to balance certain harm against uncertain benefit when considering a range of alternative strategies. The challenge is optimization – how does one achieve the maximum benefit with the minimum undesirable impact? Whenever a decision is couched in terms of sustainability, this should be defined for the question at hand: What attributes or conditions does the decision aim to sustain; Who is affected by actions (costs and benefits); For what period of time will actions convey benefits; At what cost ? and Who answers the first four questions? These are normative questions rooted in societal values, requiring engagement and a careful consideration of diverse stakeholders' priorities, bearing in mind that risks, benefits and costs are not borne equally, in terms of time, space, stakeholders, or demographics. Trade-off evaluation should take into account affected communities' vulnerabilities, needs and values, and how these might be impacted by remedial options. Many stakeholders who do have the time and resources to engage in the decision process have a primary focus on a single or narrow set of remedial impacts. The use of frameworks that guide stakeholders to consider the extent to which they prioritize all (rather than just a narrow subset) of the impacts to of their values can support a more balanced public comment process, less subject to single- or narrow-issue lobbying, but capturing, understanding and addressing the needs of diverse stakeholders can be challenging. Identification of risks and benefits of most interest to stakeholders can support negotiation and optimization of alternatives, but unengaged subjects, due to a lack of resources, interest, or awareness, may not have their needs and values addressed unless a special effort is made to identify and consider them. There can be fundamental disagreement between stakeholders on the desirability of various management impacts. Environmental scientists seeking to support more equitable decision making must address the embedded assumptions and limitations of tools we commonly use. These issues, and potential strategies to address them, will be discussed.

WE045
What and where are the important social impacts?
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The LCA framework has proved useful to assess social impacts for products and product systems. Still, the field can be regarded as relatively immature even after more than twenty years. Reasons for this include the great number of possible impact categories, the ambiguity inherent in many social impacts, and the esoteric nature of the impact categories for a field predominantly developed by natural scientists and engineers. The development of tools such as the social hotspot database (Benoît Norris, 2014) and the PSILCA database (Ciroth and Eisfeldt, 2017), which combines economic input output data and various data on social impact indicators, has been an important achievement in furthering the application of S-LCA. These provide a possibility for quick assessments of value chains based on limited information – however, the answers from such assessments are difficult to interpret. This work aims at a better understanding of social impact categories and identification of risk of negative social impacts. The analysis addressed three questions: Are the social and socio-economic subcategories correlated? A high degree of correlation means that a higher risk of a social impact within subcategory A in sector X in country 1 than in sector X in country 2 also implies a higher risk of a social impact within subcategory B in sector X in country 1 than in sector X in country 2. Are the risks of social impacts across industrial sectors in a country consistent? If the risks are consistent across industrial sectors in the countries, then a country with a higher risk of social impacts within sector X will also have higher risk of social impact within sector Y. Are the risks of social impacts across countries for various sectors consistent? If the risks are consistent across countries, it shows that if country A has higher impacts than country B in one subcategory, it will also have higher impacts in another subcategory. The assessments showed strong correlations between the variables. This means that social hotspots can be anticipated from knowing which countries processes will take place. It also means that there is a large need for specific data for social LCA as generic data are most often on the country level and does not provide details to better understand the production system under scrutiny.

WE046
Current state and methodological challenges of life cycle sustainability assessment: a review
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Life Cycle Sustainability Assessment (LCSA) is a life cycle oriented tool that encompasses environmental, economic and social aspects. Despite the growing interest in LCSA methodology in recent years, it remains unclear on how to apply it. Therefore, the major objectives of this paper is: (1) to carry out a systematic review of the scientific literature (until 2018); (2) to identify how LCSA is currently addressed, including the methods used to conduct it, and; (3) to identify the main challenges for a wide implementation of LCSA. The review was conducted according to the guidelines protocol proposed by Pullin and Stewart [1]. The studies (139 peer-reviewed articles) were selected considering research questions and keywords that focus on the LCSA development. These publications were categorized as case studies (45), reviews (33), methodological development (32), mixed approaches (22) and viewpoints (7). Mixed approaches (articles that propose LCSA methods and apply it to case studies) and case studies where classified considering three approaches: (1) LCSA = LCA + LCC + SLCA, LCSA as the sum of Life Cycle Assessment (LCA), Life Cycle Costing (LCC) and Social Life Cycle Assessment (SLCA), (2) LCSA as a new assessment, and (3) other approaches. The 'LCSA =new' approach requires the definition of a single inventory to assess environmental, social and economic impacts [2]. The category 'other approaches' include LCSA as the sum of eco-efficiency with SLCA or LCSA as the sum of LCA with socioeconomic analysis, as well as the dynamic LCSA. This review shows that the use of LCSA in case studies has been growing in recent years, being the 'LCSA = LCA + LCC + SLCA' the most applied approach. However, further developmment is required to better understand the interactions between the environmental, economic and social dimensions. The review also shows that the main challenges for LCSA are the need to ensure consistency between the different system boundaries, the lack of databases for the assessment for social and economic data, definition of impact categories, methods to conduct sensitivity and uncertainty analysis, and strategies towards the communication of results. References [1] Pullin AS and Stewart GB. 2006. Guidelines for Systematic Review in Conservation and Environmental Management. Conservation Biology20, 6:1647-1656. [2] Kloepffer W. 2008. Life cycle sustainability assessment of products. International Journal of Life Cycle Assessment13, 2:89-94.

Organic Micropollutants in Urban Waters (P)

WE047
Adsorption of ionizable pharmaceutical compounds onto microwave-hydroxide functionalized biochar
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Wild plums are among the most common fruits in Serbia, and kernels are generally disposed of as waste. A new generation of functionalized biochar (WpOH) was prepared from wild plum kernels by simultaneous pyrolysis and microwave KOH functionalization, and used for ionizable pharmaceutical (naproxen (NPX)) removal from water. NPX belongs to the aryl acetic acid group of non-steroidal anti-inflammatory drugs and has been detected all over the world, at up to 2.3 μg/L in effluent from wastewater treatment plants, confirming that NPX, and many others similar compounds, are not effectively removed by conventional treatment. NPX is now on the Global Water Research Coalition (GWRC) priority list and is already a more common NSAID than diclofenac in Scotland (due to a significant increase in dispensing rates). Characterization of synthetized biochar was carried out using pH_{pzc}, SEM/EDX, BET, FTIR, XRD and the governing adsorption mechanisms were thoroughly studied. The microporous biochar possessed a large specific surface area (601.9 m^2/g) and micropore volume (0.231 cm^3/g). The development of porosity during the KOH microwave activation can be explained by the diffusion of metallic potassium (formed during the pyrolysis process) into the internal structure of the lignocellulosic material. This had an impact on existing pores (causing spreading) and acted to create new ones. A pseudo second-order kinetic model described the reaction kinetics and the Langmuir isotherm gave a best fit to the results. The maximum adsorptive interaction (73.137 mg/g) occurred between pH 5 and 7, through electrostatic attraction (the main interaction mechanism) for the negatively charged NPX species and the positively charged biochar surface. The mass transfer analysis showed that the adsorption of NPX first took place through surface reactions with functional groups until these were saturated; thereafter, NPX diffused into the biochar porous structure, adsorbing within the inner pores. FTIR and XRD revealed that hydrogen-bonds, π–π and n–π electron-donor-acceptor interactions also played a role. The results highlighted the high potential for the use of wild plum kernels to produce biochar and its efficiency in removing emerging contaminants such as pharmaceuticals from contaminated water.

WE048
Concentrations of Polybrominated Diphenyl Ethers, Hexabromocyclododecane, and Perfluoroalkyl Substances in Landfill Leachate from Ireland
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Introduction This study tests the hypothesis that past disposal to landfill of waste will have led to significant contamination of landfill leachate with polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD), and perfluroalkyl substances (PFASs). To do so, we report concentrations of such contaminants in samples of leachate from landfills in the Republic of Ireland. Materials and methods Leachate was collected between June and November 2017 from 40 landfill sites across the Republic of Ireland. At least 1 sample of leachate was

collected from each landfill studied with 46 samples collected in total. Cleaned leached extracts were subjected to GC-MS for determination of PBDEs, with LC-MS used to determine concentrations of alpha-, beta- and gamma-HBCDD, and PFASs. **Conclusions** Our data reveal substantial inter-landfill variation in concentrations of our target BFRs and PFAS detected in leachate from Irish landfills. Likely due to their greater aqueous solubility, concentrations of PFASs generally exceed those of PBDEs and HBCDD. Interestingly, the median concentration of perfluorooctane sulfonate (94 ng/L) only just exceeded that of one of its replacements, perfluorobutane sulfonate (79 ng/L). Concentrations detected of PFASs in this study are of similar magnitude to those reported recently for Australian and Canadian landfill leachate, with those of PBDEs also falling within the range of previous studies elsewhere in the world. Further work in this project will examine the influence on contaminant concentrations in leachate of both landfill characteristics (e.g. time window over which waste was accepted at the landfill, whether landfill is lined or unlined etc.) and leachate properties (e.g. organic carbon content, pH etc.). In addition, in the second phase of this project, 10 of the landfills whose leachate was analysed here will be studied in more detail, with concentrations of target BFRs and PFAS measured in air and soil in the vicinity of the landfill, as well as in samples of groundwater potentially impacted by landfill leachate (e.g. for unlined landfills).

WE049

CWPharma - case study about active pharmaceutical ingredients emissions and sources in Estonia

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Emissions of active pharmaceutical incridients (APIs) to the water environment is a rising concern around the world. Residues of APIs contaminate the water and marine life of the Baltic Sea, therefore presence of these residues in enironment is of great concern. Information about emissions, sources and environmental levels of APIs is still insufficient. During this study the consumption data of both human and veterinary medicines was collected. For estimation of emissions to the water environment targeted sampling campaigns on potential sources. 65 active pharmaceutical ingredients were analyzed, samples were taken from surface water, fish farm and municipal wastewater treatment plant effluent, influent. Almost half of the analyzed APIs were under 2 ng/L in surface water and fish farm samples. Highest concentrations in surface water were detected for Bisoprolol (158 ng/L) that is used to treat high blood pressure and heart failure and for an anti-inflammatory agent Mesalazine (71 ng/L) from the effluent of fish farm. 91 % of the analysed API residues were found from the waste water treatment plant effluents. The concentrations ranged from as low as 20 pg/l up to 147 Âg/L. Wastewater treatment plants are the first defensive line between aquatic environment and emissions of APIs. During this study consumption data, potential sources and levels of environmental emissions as well as an efficiency of present wastewater treatment technology (activated sludge process with biological nutrient removal) will be analysed for developing the measures for pollution reduction. **Acknowledgements** Study is carried out as a part of p_Clear waters from pharmaceuticals – CWPharma" project funded by the European Union Interreg Baltic Sea Region program along with co-financing from Estonian Environmental Investment Centre.

WE050

DETECTION OF SYNTHETIC POLYCYCLIC MUSKS IN SEDIMENTS OF THE UMGENI AND MSUNDUZI RIVERS IN KWAZULU-NATAL, SOUTH AFRICA

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Synthetic polycyclic musks (SPMs) are emerging contaminants mostly arising from personal care products where they are widely used as fragrances in cosmetics, soaps, shampoos, detergents, deodorants, lotions, perfumes, food, cleaning products, cigarette additives and other products. They are ubiquitous contaminants that are lipophilic in nature. They are widely present in environmental samples, for example, in water sources, because they are continually released into the aquatic environment, and, due to their lipophilicity, they tend to be persistent and accumulate in sediments, sludge, water, soil, aquatic life, air, marine mammals, human breast tissue and breast milk. These SPM compounds are considered endocrine modulators as they affect fish embryo and larva stage development; are carcinogens and impair fertility in rats. An investigation of these SPMs was prompted because they are observed to be only partially eliminated in sewage treatment plants, and there is an increasing trend in their production volume and usage. Currently, there is a paucity of data from Africa on these substances, since they are not commonly monitored. There is no information on their concentrations and distribution in KwaZulu-Natal Rivers, especially the two rivers of interest, namely, the uMgeni and Msunduzi rivers. These rivers have anthropogenic, agricultural and industrial activities, as well as return flow from different wastewater treatment plants, dams and tributaries along their courses. Sediment samples were collected at eighteen sites along the courses of these two rivers for the summer season in January 2015. The sediment samples were dried in a clean drying room and partitioned into different mesh sizes with a sieve. Extraction of the sediments was carried out with the aid of an ultrasonic bath and hexane/acetone (1:1) as the extracting solvent. The extracts were cleaned by using silica gel and anhydrous sodium sulfate. Quality assurance and quality control measures were ensured. The sediment samples were analyzed for SPMs by means of gas chromatography-mass spectrometry. The method has a good recovery of 78 to 120% with an intra-day and inter-day percentage relative standard deviation (%RSD) observed to be < 7%. Results obtained indicate the presence of the SPMs of interest in the sediment samples of the uMgeni and Msunduzi Rivers except at Camp Drift where the concentration is observed to be lower than the limit of detection.

WE051

Does chiral inversion change the ecotoxicity of pharmaceuticals?

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Wastewater treatment plants are a significant pathway of pharmaceuticals to the aquatic environment. Many commonly used pharmaceuticals, including some non-steroidal anti-inflammatory drugs (NSAID), are chiral chemicals and their enantiomers can exhibit different biological activity. However, very few studies have considered the potential ecotoxicological differences of pharmaceutical enantiomers. Recent studies have shown apparent chiral inversion of pharmaceuticals during wastewater treatment, with higher concentrations of (R)-naproxen detected in wastewater effluent despite naproxen being manufactured as pure (S)-naproxen. The overall project aims to determine if biological wastewater treatment can increase the ecotoxicity of chiral pharmaceuticals. To assess the ecotoxicological differences of NSAID enantiomers, (R) and (S) enantiomers of naproxen, ibuprofen, ketoprofen and flurbiprofen were run in a battery of bioassays using bacteria, algae and fish cells. While many of the enantiomer pairs had a similar effect in the bacterial toxicity screen, the (S) enantiomer of flurbiprofen was more toxic than the (R) enantiomer. The opposite was observed in the combined algae test, where (R)-flurbiprofen had a greater effect than the (S) enantiomer. Further, several of the enantiomer pairs showed differing ethoxyresorufin-O-deethylase (EROD) activity in fish cells. This study shows there are differences in effect for some pharmaceutical enantiomer pairs and indicates the importance of considering enantiomers when assessing the risk of pharmaceuticals to the receiving environment.

WE052

Estimation of annual active pharmaceutical ingredients emission from small municipal waste water treatment plant in Poland

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Pharmaceuticals and their active ingredients (APIs) in the environment have become one of the most emerging issues nowadays. Lots of efforts are put to estimate both the environmental levels of APIs as well as their ecotoxicological impacts on living organisms. In this study the estimation of annual loads of APIs from small MWWTP located in Blonie, Poland is presented. 24 h flow proportional composite effluent samples were collected in two seasonal campaigns –autumn 2017 and spring 2018. Totally 65 and 63 compounds were detected by UHPLC-MS/MS method in the samples in autumn and summer respectively, representing different therapeutic groups e.g. antibiotics, antihypertensives, non-steroidal anti-inflammatory drugs (NSAIDs), betablockers and lipid-lowering agents. Twenty substances were analysed but not quantified (< LOQ) in autumn and 26 in the summer. The most abundant compounds in effluents (with concentrations above 1 mg/L) were: Carbamazepine < Fenbendazole < Mesalazine < Telmisartan < Gabapentin < Diclofenac < Allopurinol < Nebivolol < Ciprofloxacin < Ivermectin in autumn season and Ivermectin < Diclofenac < Carbamazepine < Gabapentin < Telmisartan in summer season, respectively. For substances with concentrations The annual loads for compounds with the highest concentrations were: **API Concentration in effluent [ng/L] Annual load [kg/y] Autumn Spring** Ciprofloxacin 1241435,05 92,20 1344,509 Nebivolol 32037,72 3,14 34,699 Allopurinol 12565,28 < 13,72 13,614 Diclofenac 8462,28 1703,91 10,843 Telmisartan 1963,48 6493,64 8,526 Gabapentin 2368,85 5822,67 8,304 Carbamazepine 434,72 2485,60 2,920 Mesalazine 1906,61 < 1,58 2,066 Fenbendazole 1399,23 54,10 1,569 Ivermectin < 177,45 1104,42 1,184 The variation in the API concentrations was observed in this study. The overall emission of 10 selected compounds was estimated to be at least 1 kilogram per year from single MWWTP. *Acknowledgement – Research was performed in the framework of EU's Interreg Baltic Sea Region project "Clear waters from pharmaceuticals – CWPharma" under WP2 "Comprehensive status of pharmaceuticals - improved knowledge on consumption, emissions, environmental levels and risks of pharmaceuticals".*

WE053

Ethylparaben adsorption onto activated carbon

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There are lots of environmental issues but the water resources quality always deserves attention, considering its essentiality to life maintenance. With the industrial and urban growth, a large number of hazardous chemicals are detected in the water bodies due to the lack of proper wastewater treatment systems. This practice may affect the human life and aquatic ecossystems. Over the years, different organic compounds have been classified as endocrine disruptors and a great number of scientific studies have suggested that parabens could interfere negatively on endocrine and hormonal system of different species. Many studies in the literature report the presence of parabens in water bodies around the world. Thus, the necessity of pursuing alternative technologies to mitigate the environmental impact caused by toxic organic compounds is evident. In this context, this work evaluated the use of adsorption as an alternative technology to the paraben contaminated water treatment. Among other tests a coconut shell activated carbon was capable of removing up to 90% of ethylparaben, which is one of the most used parabens for preservation of personal care products and food.

WE054

Exploring the occurrence of emerging organic contaminants in Nigerian aquatic environments through a combined target and non-target screening approach

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The occurrence of emerging organic contaminants, e.g., pharmaceuticals, personal care products, and artificial sweeteners, has increasingly gaining scientific and public attention due to their ubiquitous presence in the aquatic environment and their potential of causing harmful effects on ecosystems. Research on the occurrence and distribution of emerging organic contaminants in Africa so far has been relatively scarce. In this study, we aimed at addressing this knowledge gap by exploring the occurrence of these contaminants in various types of waters including surface water, well water, tap water, and effluent samples collected from different geographical regions in Lagos, Nigeria. Both target and non-target analysis were performed to enable quantification and semi-quantification of the detected emerging organic contaminants. All samples were analyzed by direct injection on an UHPLC-Orbitrap-MS/MS operated under both positive and negative electrospray ionization modes. In total, 366 compounds were identified using the online database mzCloud, including among others pharmaceutically active ingredients, personal care products, and industrial products. Level 1 identification confidence was achieved for 31 compounds for which reference standards were available and level 2 was achieved for the rest. In the quantitative analysis of 38 target compounds, 18 were detected, including both parent compounds and metabolites. For the compounds detected in common between target and non-target analysis, the quantitative results using concentrations and the semi-quantitative results using peak areas are fairly consistent, indicating a high reliability of our non-target approach. In general, acetaminophen, caffeine, and sulfamethoxazole were the organic contaminants detected at the highest average concentrations (up to 145 mg/L for acetaminophen in the samples collected from Lagos Lagoon). Wastewater and lagoon water contained the most organic contaminants with noticeably high concentrations. In addition, seven contaminants were found in the tap water, with a concentration ranging from 0.062 μg/L for acesulfame to 103 μg/L for caffeine. These findings point to a high potential of hazards for the population and ecosystems in the regions.

WE055

Extraction, isolation and characterisation of selected benzodiazepines in wastewater

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Pharmaceutical pollutants entering the aquatic environment have become a growing environmental concern. These pharmaceuticals are unique pollutants because of their special characteristics and behaviour that cannot be simulated with other organic pollutants. The untreated wastewater effluent that contains pharmaceuticals poses considerable threat to the aquatic ecosystem because of the negative effects of non-target organisms in the water. Recent years have seen a growing concern about the benzodiazepines, as emerging pollutants, and their effects on the aquatic environment. These compounds are nowadays widely detected in sewage wastewater. It is important to increase the emphasis on the characteristics of the benzodiazepines in order to differentiate them from industrial chemical compounds. In this study various solid phase extraction techniques have been employed focussing on isolation of benzodiazepines in wastewater matrices. Employing this methodology has shown improved detection and analysis of clonazepam and lorezapam as benzodiazepines.

WE056

Fate of TBBPA in rhizosphere of wetland plants: Removal under shifting oxic-anoxic conditions

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Tetrabromobisphenol A (TBBPA) is a brominated flame retardant used worldwide. Due to its high lipophilicity and poor water solubility, TBBPA tends to accumulate in the environment. TBBPA has been detected in various environments such as air, sewage sludge, sediment and soil, and even in organisms such as mussels and birds. Therefore, the need for TBBPA removal from the environment is indispensable. The degradation of TBBPA has been examined in various studies and it has been found, that a full metabolization takes places neither under oxic nor under anoxic conditions. A sequential shift of oxic conditions seems promising, just as it is naturally occurring in the rhizosphere of constructed wetland plants. The aim of this study is therefore to establish a method to simulate the shifting areation conditions of rhizosphere decoupled from other rhizosphere effects and thereby achieve a complete removal of TBBPA. The model plant for this study, *Phragmites australis*, was sampled from a field constructed wetland and cultivated in a greenhouse. To spatially quantify the radial oxygen loss (ROL) of the plant roots, quantitative imaging by luminescence sensing of a planar oxygen optode was used. Artificial roots were built from ultrafiltration membrane and connected to an air pump. The oxygen flow rate of the artificial roots was adjusted to meet the plant ROL, also by measuring with the planar optode. Within the next months, the artificial roots will be used in a TBBPA removal study. Removal at diurnal shifting areation conditions will be compared to removal at constant oxic conditions, removal without oxygen supply and a sterile control. The artificial roots will be placed in sediment sampled from the constructed wetland saturated with wastewater in closed systems. ^{14}C-labelled TBBPA will be used to trace the substance and its metabolites. Using liquid scintillation counting (LSC) and ultra performance liquid chromatography-mass spectrometry (UPLC-MS), the formation of metabolites and mineralization will be determined. This work is part of the collabrative project of the National Natural Science Foundation of China (NSFC) and the European Union (Grant No. 31861133003).

WE057

Finnish Watch list - The screenings results 2015-2018 in surface waters

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We present results of Finnish surface water screening of watch list substances (EC implementing decision 2015/495). Samples were collected from 9 sites according to the directives demand during 2015–2018. The sites presented varying types of pressures from urban to agricultural while some of the sites represented background conditions. The sites included 7 rivers, a lake near city of Tampere and a pipe from Lake Päijänne, which water is used to produce drinking water for Helsinki metropolitan area. The substances included 3 estrogens, 3 macrolide antibiotics, diclofenac, 5 neonicotinoids, 3 other agricultural pesticides, BHT and ECHM. A requirement for quantification limit (LoQ) for analysis was given in the EC decision for each substance or group of substances basing on the compound predicted no effect concentration (PNEC). The samples were analysed in a laboratory of Eurofins and the LoQ requirements were fulfilled. The concentration of estrogens in Finnish surface waters have never before been analysed using as low LoQs. The synthetic estrogen 17-α-ethinylestradiol (EE2) was detected in concentration of 0.035 ng/L, which is its PNEC level (Loos et al. 2018), in 3 sites. Estrone (E1) was detected in several sites but its PNEC was not exceeded. Diclofenac was detected widely and its concentration was above its PNEC of 50 ng/L (Loos et al. 2018) in several river samples. The occurrence of diclofenac, EE2 and macrolides, which were detected in trace levels in several sites, were strongly associated to effluents from waste water treatment plants (WWTP). Their concentration fluctuation in time was affected by the volumes of diluting water (river discharge). In 2018, some spills in up-stream WWTPs during low discharge period caused high peak concentrations. Neonicotinoids clothianidin and thiamethoxam were detected frequently in the rivers under agricultural pressure, but their concentration did not exceed PNEC levels. Over half of the compounds were not detected at all: e.g. estradiol, which is estimated to enter into sludge in WWTP, the pesticides which are not used/usage is very limited (acetamiprid, imidacloprid, tri-allate, oxadiazon, methiocarb) and BHT, which sources are unknown. EHMC was detected in a sample. The launching of watch list accelerated the development of analytical methods. Results show that diclofenac and EE2 may occur in possibly harmful concentrations in Finnish surface waters under urban influence and their monitoring should continue.

WE058

Hydrolysis of modern antifouling biocides: Zinc Pyrithione and Zineb

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The environmental fate of two metal-organic biocides, zinc pyrithione (ZnPT) and zineb, is difficult to assess, which is partially due to i) the difficulty to dissolve the respective compounds, ii) questionable stability of the compounds in water or solvents, and iii) their ability to exchange their metal ion in a process called transchelation. This transchelation process, combined with an inability to dissolve in common solvents, make them difficult to analyze. In this study, we aimed to investigate how these complexes behave when released into the aquatic environment. Therefore, ZnPT and zineb were suspended in natural seawater, artificial seawater, sterilized seawater (all salinity about 1.2%) and in deionized water. In these samples, ZnPT, zineb and their transformation products were monitored using an LC-MS/MS, in order to study the ongoing transformations in each sample. In this experiment, it became clear that ZnPT and zineb themselves were not detected using LC-MS/MS. The two transformation products monitored for ZnPT were the sodium pyrithione transchelate and dipyrithione. Sodium pyrithione is found in all samples. It was formed mainly in the deionized water, where it reaches a plateau. However, in artificial seawater and natural seawater, the sodium pyrithione was found to hydrolyze after initial formation. Dipyrithione has been detected as a transformation product of sodium pyrithione. Formation and degradation of dipyrithione follows the sodium pyrithione in the sterilized brackish water, brackish water and deionized water. However, dipyrithione does not show similarities to sodium pyrithione in the artificial seawater, where dipyrithione is formed at a much slower rate and does not show any dynamics resembling those of sodium pyrithione. For zineb, only a single transformation product could be monitored, which is ethylene bis-isothiocyanate sulfide (EBIS). This product was readily detected and no differences between the treatments could be observed. Moreover, no degradation of EBIS could be detected, meaning it might be a stable transformation product. This study shows, not only, that the transformation products should be included during environmental monitoring of ZnPT and Zineb, but, also, that the environmental fate of these metal-organic biocides depends on specific environmental conditions.

WE059

Identification and quantification of polybrominated dipheyl ethers (PBDEs) in environmental samples using gas chromatography coupled to Orbitrap mass spectrometry

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Polybrominated diphenyl ethers (PBDEs) are a group of organobrominated contaminants that inhibit or suppress combustion in organics material, and due to these properties have been widely used since the 1970s as flame retardants. Most PBDEs resist degradation, and bioaccumulate in environment, and food chains, and can be transported through air, and water over long distances. Many PBDEs have been identified as toxic, with links to cancer, and endocrine disruption and the use of certain PBDE have been banned. There are many analytical challenges to consider when developing a GC-MS method for the analysis of PBDEs, especially related to the active nature of high molecular mass PBDEs (e.g. BDE-209), and to the large number of compounds, resolution, and peak symmetry requirements (e.g. chromatographic separation of BDE 49 and BDE 71 can be challenging in complex environmental samples). This work demonstrates the use of high resolution accurate mass GC-Orbitrap technology for the targeted analysis of 27 PBDE native congeners in air, ash, sediment, and sludge samples using a sensitive, fast, and robust high throughput method. Sediment, filter dust, air, and sludge samples were assessed for their PBDE content. In the sludge samples analysed, the predominant PBDE congeners identified were BDE-209, 47 and 99, where as in the filter dust samples BDE-47, 119, 99, 53 and 183 were prevalent. This preliminary results offers excellent selectivity, and sensitivity for the analysis of PBDEs in complex samples. Moreover, the use of high resolution to achieve selectivity in difficult matrices, and the mass accuracy obtained allows for unambiguous identification, and elemental composition confirmation of chemical contaminants.

WE060

Leaching of diflufenican and transformation products in the urban environment

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In the urban environment, courtyards, driveways, tiled squares, and other places are sprayed with persistent, pre-emergent herbicides to avoid plant growth. Extensive use of such herbicides has resulted in serious contamination of groundwater in Denmark (e.g. dichlobenil transformation to the metabolite 2,6-dichlorobenzamide (BAM)). We are presently focussing on the herbicide diflufenican that, along with glyphosate (Keeper-L), is used in paved areas. However, its product registration was based on the data obtained from agricultural soil that typically presents much higher organic matter and sorption capacity. Thus, we hypothesized that in urban applications, diflufenican and its transformation products can more easily end up contaminating both surface water (via stormwater run-off) and groundwater. Two separate outdoor experiments, subject to natural rain and meteorological conditions, have been set up to separately study both emission routes. 1) to study surface runoff and leaching from paved surfaces, big steel trays with a depth of 12 cm were filled with sand/gravel overlaid with either concrete tiles or standard gravel paving forming a full-section of 1 m^2. 2) to compare the potential leaching to groundwater from five different soil types, a multicolumn lysimeter (21 columns, each 50 cm long and 15 cm in diameter) was set up. A sandy and a loamy agricultural soil, and three types of commercial gravels found in the Danish stores were assessed. Both experimental setups were sprayed with Keeper-L following the manufacturer indications. Leachate from the systems was collected up to 1-year period and analyzed by HPLC-MS/MS. The two paved surfaces leached diflufenican immediately in the first rain event, both through surface run-off and infiltration. Concentrations were as high as 11 μg/L in the run-off and leachate, while both known aerobic transformation products (AE-O and AE-B) were detected 7 days after the spraying. Different retention patterns were observed depending on the pavement material. The leachate from the outdoor multicolumn lysimeter revealed no leaching of diflufenican within a 1-year period. Nevertheless, concentrations up to 1.6 μg/L of both transformation products, AE-B and AE-O, were measured for the gravels but not for the agricultural soils. The different gravels showed also different TPs formation and retention patterns. In sum, we may be emitting diflufenican and its TPs to urban waters.

WE061

Micropollutants present in wastewater emitted in the Baltic Sea catchment - an overview

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Hazardous substances are recognized as a threat to waterbodies in the Baltic Sea region and the EU, which is acknowledged in the EU's water legislation. However, the importance of municipal WWTPs as entry routes for these substances is in many cases neither well studied nor acknowledged in current wastewater policy. There is a lack of knowledge regarding which chemicals are present in wastewater and their observed negative effects. The lack of knowledge leads to a lack of policy action as currently available data do not suffice to establish requirements for acceptable loads or removal efficiencies of micropollutants entering MWWTPs. In this study, we aim to map micropollutants measured in wastewater emitted in the Baltic Sea catchment. A literature search for scientific publications reporting concentrations of any micropollutants in effluents was performed. In addition, data were compiled from national monitoring databases and project reports. National monitoring contacts were approached directly and asked for publically available monitoring and screening data. The type of chemicals typically measured, detection frequencies and detected levels, data availability for different Baltic Sea nations were analyzed and data gaps highlighted. We found data for ca 830 unique substances measured in ca 25 000 samples of effluents emitted in the Baltic Sea catchment. In approximately half of the ca 25000 recorded samples, the concentration of the analyzed substance was above the analytical limit of the method (detection limit or quantification limit). The type of compounds with highest detection frequency were organophosphates, sweeteners, fluorescent whitening agents and components in household products. In absolute numbers, pharmaceuticals was the most commonly detected type of substance, although ca 50% of these analysis were reported as non-detects. Screening campaigns are needed in particular in the eastern Baltic Sea countries and Denmark in order to assess national differences and better understand the role of MWWTPs as filters between urban and aquatic environments in the Baltic Sea region.

WE062

Modelling of pharmaceutical loads to the Baltic Sea - A case study in Southern Finland

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Active pharmaceutical ingredients (APIs) are detected globally in surface waters and in a variety of other matrices. The knowledge on the API load to the Baltic Sea environment is currently inadequate, and the spatial coverage of information produced in screening campaigns and surveys is sparse. The objective of this study was to estimate the overall emissions of selected APIs into the Baltic Sea as well as estimating the concentration of these substances in the estuary area. To a large extent, APIs are emitted through human consumption of pharmaceuticals and subsequent waste water emissions. The pathway of a consumed pharmaceutical product to API emission into the environment is affected by several processes, such as metabolism, elimination in waste water treatment processes and possible elimination in the environment. In this study we estimated the loads and concentrations of selected APIs using a GIS-based model. Assuming the consumption of pharmaceuticals is uniform within each country, the human population in each area can be seen as the driving parameter for the input of APIs into the environment. The model combines information on inhabitants and national statistics on the consumption of pharmaceuticals to calculate the API usage in each grid cell. We further coupled the usage information with API-specific parameters, such as excretion rate of the active substance and removal rate in waste water treatment processes to estimate the environmental load of these substances. Furthermore, API-specific behavior, such as degradation in the environment was taken into consideration to estimate the total load of the selected substances reaching the Baltic Sea. To evaluate the impact of the processes occurring in the environment, we approximated the distance and travel time from each grid cell to the Baltic Sea. The poster presents the preliminary results for a case-study area located in Southern Finland as well as discusses the possible sources of error. The calculation method will be used in estimating the potential of different emission reduction measures. The study is part of the project Clear waters from pharmaceuticals (CWPharma), which is funded by EU's Interreg Baltic Sea Region Programme.

WE063

Modified biochar for efficient removal of pharmaceuticals from water

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Activated biochar (ACT-B) was prepared from *A. sylvestris* or cow parsley (a locally grown plant that can be a problematic weed) by modifying it with NaOH. The prepared material was characterized by several techniques, viz., scanning electron microscopy, Fourier transform infrared spectroscopy, pH_{zpc}, elemental analyzer and Brunauer–Emmett–Teller in order to gain an insight of its physico-chemical properties. The potential of ACT-B was assessed for the removal of diclofenac sodium (DF) and cephalexin (CPX), two important pharmaceuticals from water. Batch studies were first conducted and different adsorption parameters such as effect of solution pH, contact time, temperature, adsorbent dosage, adsorbate initial concentration and co-existing ions were investigated to optimize the process. The kinetic and equilibrium data were fitted into different kinetic and isotherm models. ACT-B exhibited high adsorption capacity for DF and CPX. The removal efficiency of DF and CPX was influenced by temperature and the competing ions, while the highest removal of DF and CPX occurred at normal pH. Moreover, to understand the applicability of ACT-B in real scale for the removal of DF and CPX, column studies were also carried out using different adsorbate concentrations and flow rates. Mainly, pi–pi bonding was found to be responsible for the adsorption of the studied pollutants onto ACT-B. The results of this study revealed that modified biochar has high potential in removing DF and CPX from water.

WE064

Occurrence of aromatic micropollutants and their chlorinated derivatives in wastewater and river of Taichung metropolitan area

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Pharmaceuticals and personal care products (PPCPs) with aromatic structures are likely to be transformed into chlorinated derivatives even in low-dose chlorination (0.2-0.7 mg/L). In this study, we investigated the occurrence of ten aromatic PPCPs and their 16 transformation products (TPs) in a domestic wastewater treatment plant located at the metropolitan district of Taichung, Taiwan. The wastewater and water in the river which receives the effluent were sampled (100 mL, n = 3 each) according to the hydraulic retention time in October 2018. Samples were pretreated using solid-phase extraction. The aromatic PPCPs and their TPs were determined or semi-quantified using ultra-performance liquid chromatography coupled with triple-quadrupole mass spectrometry. The dynamic ranges of the PPCPs were mostly between 2.5 ng/L to 20 µg/L with most of the quantification biases within ± 10%. Methylparaben, bisphenol A, and diclofenac were found in all of the samples at the levels ranged from tens to more than 100 ng/L. The concentrations of these three aromatic PPCPs were generally higher in the wastewater than in the receiving river water, probably due to dilution. However, unrecorded sources of contamination may have brought into the receiving river acetaminophen and propylparaben (tens ng/L), which were absent in the wastewater. The unspecified sources and physicochemical reactions may also have resulted in the positive detections of the chlorinated TPs of propylparaben and triclosan ($C_{10}H_{10}Cl_2O_3$ and $C_{12}H_6Cl_4O_2$, respectively) in the river water. The results demonstrated the occurrence of chlorinated TPs, which are more toxic than their precursor PPCPs, in the complex urban water system. Future work will be concentrated in elucidating the temporal trends of the transformation of aromatic PPCPs by observing the relative concentrations of the chlorinated TPs in the following seasons.

WE065

OPTIMISATION OF A MICROWAVE ASSISTED EXTRACTION PROCEDURE COMBINED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF TAMOXIFEN AND CYCLOPHOSPHAMIDE IN FISH TISSUES

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A serious problem today is marine pollution caused by human activities. Part of this contamination is due to pollutants that come from wastewater treatment plants due to an ineffective treatment in the elimination of certain compounds (Montesdeoca-Esponda et al., 2018). Some of them are antineoplastic compounds, which are drugs used as treatments against cancer and can cause important adverse effects if they reach the environmental waters (Parrella et al., 2014). Antineoplastic compounds have been detected in hospital effluents, wastewater treatment plants influents and effluents, river and surface waters, and sludge (Santana-Viera et al., 2016). To date, no procedure has been optimized for the extraction of antineoplastic compounds from fish tissue. In this work, we optimised a procedure for the extraction and determination of Cyclophosphamide and Tamoxifen in fish tissues by using microwave assisted extraction with liquid chromatography tandem mass spectrometry. The developed procedure was applied to the extraction and determination of these two compounds in muscle and liver tissues of to three different fish species of different levels of the trophic chain captured in the vicinity of three marine outfalls. **REFERENCES** Montesdeoca-Esponda, S., Checchini, L., Del Bubba, M., Sosa-Ferrera, Z., Santana-Rodriguez, J.J., 2018. Analytical approaches for the determination of personal care products and evaluation of their occurrence in marine organisms. Sci. Total Environ. 633, 405–425. https://doi.org/10.1016/j.scitotenv.2018.03.182 Parrella, A., Lavorgna, M., Criscuolo, E., Russo, C., Fiumano, V., Isidori, M., 2014. Acute and chronic toxicity of six anticancer drugs on rotifers and crustaceans. Chemosphere, 115, 59–66. https://doi.org/10.1016/j.chemosphere.2014.01.013 Santana-Viera, S., Montesdeoca-Esponda, S., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2016. Cytostatic drugs in environmental samples: An update on the extraction and determination procedures. TrAC - Trends Anal. Chem. https://doi.org/10.1016/j.trac.2015.08.016 \n

WE066

Profile of emerging contaminants and their transformation products in drinking water in Taiwan

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Sequential water treatment processes in drinking water treatment plants (DWTPs) may be capable of removing organic micropollutants from raw water. However, recent concerns have risen over the transformation of pharmaceuticals and personal care products (PPCPs) into chlorinated derivatives. In this study, we evaluated the removal efficiencies of ten PPCPs and the generation of their 16 transformation products (TPs) in the DWTPs in Taiwan. Raw and finished water samples (500 mL, each n = 8) were collected from six major DWTPs, which are located in northern, central, and southern Taiwan and perform similar water treatment processes. These water samples were pretreated using solid-phase extraction (SPE) with methanol elution. The extracts were concentrated and reconstructed to 1 mL with methanol before being analyzed using reversed-phase liquid chromatography coupled with triple-quadrupole mass spectrometry. The relative quantification of the PPCPs and TPs were performed by comparing their peak areas to those of diclofenac-d_4, which was spiked before the SPE. Three PPCPs and one TP were found in the water of DWTPs. While acetylsalicylic acid and methylparaben appeared in almost all of the water samples, diclofenac was found in only one finished water sample. On average, 17.6% and 27.6% of acetylsalicylic acid and methylparaben, respectively, were removed in the DWTPs. However, the highest levels of acetylsalicylic acid and methylparaben in the finished water were 31.4 and 8.1 times, respectively, more abundant than those of the lowest. A TP of acetaminophen ($C_8H_7Cl_2NO_2$) was found in both the raw and finished water of a DWTP, of which the raw water was composed of the water from a river and a reservoir. The retention of acetaminophen in the reservoir may have increased the probability of TP formation. This study demonstrated that given similar treatment processes, the source of raw water should have a decisive influence on drinking water quality in terms of micropollutant contamination.

WE067

Quantitative and qualitative analysis for emerging organic pollutants in wastewater from WWTP in Busan, Korea via target, suspect and non-target screening

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Emerging organic pollutants such as pharmaceuticals, personal care products, and pesticide are entering into water system via effluents from WWTP. Although much research has been performed about environmental pollutants in wastewater, there are many substances that are still unknown and/or excluded from regular monitoring work mainly due to the lack of analytical tools. To identify them, emerging analysis techniques such as suspect and non-target screening using HRMS required. The aim of the present study is to quantitatively analyze well-known emerging pollutants and identify less-recognized ones in effluent from WWTP via advanced analytical methods such as suspect and non-target screening using LC-HRMS(QExactvie+ Orbitrap). The effluent samples were taken from four WWTP located in Busan in October 2018 and enriched with multi-layer SPE method. Among 180 target compounds, 32 pharmaceuticals (Ampicillin, antipyrine, bisoprolol, carbamazepine, caffeine, cimetidine, clarithromycin, celecoxib, climbazole, cetirizine, diphenhydramine, lidocaine, fexofenadine, fluconazole, fluoxetine, irbesartan, lamotrigine, lincomycin, losartan, mefenamic acid, naproxen, niflumic acid, oxcarbazepine, propranolol, ranitidine, roxithromycin, sitagliptin, sulfamethoxazole, sulpiride, telmisartan, trimethoprim, venlafaxine), 5 metabolite (albendazole sulfoxide, carbamazepine 10,11-epoxide, O-desmethyl venlaxaxine, paraxanthine/theophylline, valsartan acid) and 4 Etc (azoxystrobine, benzotriazole, carbendazim, O-toluenesulfonamide/P-toluenesulfonamide) were detected in the effluents. The concentrations range was ~ 680ng/L of pharmaceuticals, ~ 123 ng/L of metabolites and ~ 368 ng/L of others. Ranitidine used to decrease stomach acid production has shown the highest concentration and detection frequency. As results of suspect and non-target screening, 19 pharmaceuticals (1-adamantanamine, amisulpride, dextrorphan, vildaglitpin, olmesartan, tapentadol, flecainide, minoxidil, dibucaine, methocarbamol, phentermine, dihydrocodeine, candesartan, escitalopram, hydrocodone, sulfapyridine, flubendazole, benzydamine, phendimetrazine), 8 metabolites (1,7-dimethyluricacid, 2-aminobenzimidazole, 2-amino-6-methylmercaptopurine, 10,11-dihydro-10,11-dihydroxycarbamazepine, 10-hydroxycarbamazepine, clopidogrel carboxylic acid, triehtyl phosphate, tropine) and 4 etc (ensulizole, citroflex 2, tris(2-butoxyethyl) phosphate, triethyl phosphate) were tentatively identified in the effluents.

WE068

Removal of ozonation products by biofilm reactors

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Organic micropollutants are emitted with the effluent wastewater. They thus endanger surface water quality and drinking water resources. However, they can be removed by ozonation. While ozonation appears to generally work and can be conducted with acceptable costs both for wastewater and for drinking water, it often does not destroy the compounds completely, but it oxidises them. Thus from diclofenac which can be removed easily by ozone, 6-9 ozonation products are known, while for other compounds (macrolide antibiotics, venlafaxine, tramadol) the main ozonation products are the respective *N*-oxides. To avoid these compounds are emitted due to the ozonation, a successive biological treatment based on biofilm reactors is discussed without a clear idea which biofilm reactor might be used: different sandfilters, fluidised bed filters, bilogically activated activated carbon filters, pond systems, or moving bed biofilm reactors (MBBRs). All of the tested ozonation products were degraded faster in the incubations with biofilm than without. However, the reaction kinetics were diverse: While most compounds were degraded following first order kinetics, some were degraded by zero order. Both concentrations of the same ozonation product were degraded following the same order, though the reaction rates differed. This might be due to the fact that these experiments were conducted with relative thin biofilms and saturation effects might indicate for Michaelis Menthen kinetics. Especially concerning the *N*-oxides the authors were suspicious that the parent compounds of the N-oxides might be re-formed, but this was not the case. Ozonation products can be removed by biofilm reactors such as MBBRs, whether or not that approach is feasible depends on how much biomass can be stabilised downstream of the ozonation reactor.

WE069

Removal of trace substances using a modular WWTP - evaluation of the purification performance through different treatment options.

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In the course of industrialization more and more chemicals are being used. Nowadays in industrial processes, agriculture, and private households, chemicals are indispensable. As a result, they are widely dispersed in the environment. Wastewaters in particular are a significant source for so called emerging contaminants – substances which are poorly removable during traditional wastewater treatment steps and are persistent in the environment. The aim of the project AWAREGIO is to open up new opportunities especially for small enterprises through the development of modular wastewater treatment processes for the re-use of water, wastewater based nutrients and energy in agriculture, fish farming and drinking water substitution. For this, a modular pilot plant has been constructed, consisting of a primary treatment coupled to an anaerobic reactor and a moving bed biofilm reactor followed by four different secondary treatments arranged in parallel (soil filter, UV treatment, ultrafiltration and reverse osmosis). With regards to the water re-use aspect of the project, treated water from the soil filter, the UV treatment and the ultrafiltration is used for aquaponics (a combination of plant and fish cultures). Monitoring of the removal efficiency of organic trace substances is an essential part of the evaluation for water reuse. Grab and passive sampling, as well as bioassays, have therefore been applied for this purpose. In general, the more traditional treatments including the primary treatments and the soil filter appeared to be insufficient for the removal of several of the chemicals. We will present in our poster the analyses of the effluents of the different treatment steps.

WE070

Screening of pharmaceuticals in Motala ström river basin district in Sweden

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The occurrence of active pharmaceutical ingredients (APIs) in the environment is of global concern. One of the aims within the EU-project Clear Waters of Pharmaceuticals (CWPharma) is to get a more complete and reliable picture of the extent of contamination by pharmaceuticals in the Baltic Sea. Data on veterinary and human consumption of selected APIs has been collected to be used in environmental risk assessment and modelling. Mapping of environmental levels has been performed in selected river basin districts in the Baltic Sea Region covering about 200 field measurements during winter 2017 and early summer in 2018. Sampling in Sweden has been performed in the river basin district of Motala ström. Water has been collected in four lakes; Vättern, Boren, Dovern and Glan, as well as in two water courses; Stångån and Svartån. In the receiving estuary of Bråviken bay both water and sediment samples were taken. Included were also pharmaceuticals from two major hospitals and three municipal waste water treatment plants (MWWTPs) in Motala, Linköping and Norrköping. Analyses were performed both on influent, effluent and sludge from the MWWTPs and on soil samples in fields where sludge has been applied. About 60 of 75 substances were detected representing e.g. antibiotics, antihypertensives, non-steroidal anti-inflammatory drugs (NSAIDs), betablockers, antidepressants and lipid lowering agents. The results will be used to identify priority measures within the Baltic Sea Region that need to be addressed to reduce the load of APIs into the Baltic Sea. \n

WE071

Seasonal concentrations of nevirapine in wastewater at a wastewater treatment plant in Cape Town

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Emerging pollutants (EPs) are defined as substances that have been detected, which is not included in the routine monitoring programmes at the EU level, and whose fate, behaviour and ecotoxicity effects are not well understood. Pharmaceuticals used in the treatment of HIV, known as anti-retrovirals, are becoming prevalent and there is a need to quantify these pollutants and minimise any adverse affects to aquatic and human health. Nevirapine (NVP) is commonly used in the anti-retroviral treatment of HIV infection. It is known as a non-nucleoside reverse transcriptase inhibitor of the dipyridodiazepinone class, commonly used to minimize viral resistance. This study reports the isolation and chromatographic characterisation of NVP in wastewater samples, with concentrations ranging between 250 to 500 ng/L. Further evaluation of the impact of NVP on the aquatic ecosystem was also considered and reported in this study.

WE072

Stereoisomer-specific occurrence and fate of HBCD and TBECH in different wastewater treatment systems in Hong Kong

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The occurrence and fate of 1,2,5,6,9,10-hexabromocyclododecane (HBCD) and 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH), two chiral brominated flame retardants (BFRs) including sixteen different stereoisomers, were investigated in 4 wastewater treatment plants (WWTPs) featuring diverse treatment processes in Hong Kong via a 2-year sampling campaign. Mass balance analysis revealed that: 34.3 g of HBCD and 3.68 g of TBECH daily entered the 4 WWTPs, with sludge carrying much higher amounts than effluent after wastewater treatment; larger quantities of these BFRs were released in summer; effective HBCD removal was achieved via biodegradation rather than sludge sorption, while both chemically enhanced primary treatment and secondary treatment yielded high TBECH elimination (>90%). For HBCD diastereomers, α-HBCD (54–75%) predominated in all of the samples, with its proportions increased in effluent compared with influent/sludge; while for TBECH diastereomers, α- and β-TBECH (72.3–84.4% in total) were the predominant species, with a proportional shift from the latter to the former diastereomer mostly observed between influent and effluent/sludge. A faster biodegradation of β- and γ-HBCD than α-HBCD as well as β- than α-TBECH might account for this changing pattern. This is the first study reporting the enantiomer-specific behavior of chiral BFRs during wastewater treatment processes. A preferential elimination of (+)-α- and (+)-γ-HBCD and E_2-β-TBECH occurred consistently after biological treatment, likely due to microbially mediated degradation.

WE073

Temporal and spatial analysis of pharmaceuticals in tropical freshwater ecosystems

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Anthropogenic activities have strongly modified aquatic ecosystems. Among agents of global change, the concern about synthetic chemicals has grown. One example is pharmaceuticals, the consumption of which is increasing around the world. Pharmaceuticals are often not completely removed in sewage treatment plants; therefore they are detected in aquatic matrices worldwide. Another route for pharmaceuticals to the environment is the incorrect disposal of medicines. Pharmaceuticals can be harmful to ecosystems even in low concentrations. Therefore, knowledge about the occurrence of these compounds in aquatic environments is needed. In Brazil, occurrence and fate of pharmaceuticals in the environment are still poorly studied. This study was designed to analyze some of the most consumed pharmaceuticals in water samples from different freshwater systems in Brazil. Three water reservoirs (Chapéu D'Uvas, Funil, and Curuá-Una) in different parts of Brazil were studied during one season with five sampling points in each reservoir. Chapéu D'Uvas (Minas Gerais state) is a reservoir used mainly for water supply purposes, and is located at the head of a major river. It does not have any significant sources in its catchment area. The Funil reservoir (Rio de Janeiro state) is located in a densely industrialized and urbanized region. The Curuá-Una reservoir (Pará state) is located in the Amazon region, where there are small communities with no sewage treatment. In addition, River Paraibuna, which flows in to the Chapéu D'Uvas reservoir passes through a large city, was studied in a temporal approach over six seasons with four sampling points downstream of the city. All water samples will be filtered and analyzed with UHPLC-Orbitrap-MS/MS using direct injection. With this approach we can test the effect on environmental concentrations of a municipal law from 2016, prescribing drugstores to receive overdue or unused medicines for correct disposal. For all sampled water reservoirs and the river, this will be the first assessment of pharmaceutical occurrence in Brazil, where currently pharmaceuticals are not part of water quality guidelines. This study will also provide information that may support Brazilian decision makers to regulate discharge of these compounds to the environment.

WE074

The fate of pharmaceuticals in river Vantaanjoki and in the Helsinki coast

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Current knowledge on pharmaceutical loads, occurrence and environmental fate, especially in northern European conditions, is sparse. More information about the concentrations of pharmaceuticals is needed for knowledge-based risk assessments and the prioritization of substances as well as for justifying possible risk reduction measures. We conducted a pharmaceutical screening study in River Vantaanjoki, located in southern Finland and running to the Gulf of Finland in Helsinki. The river basin area is about 1 700 km^2 with around one million inhabitants (1/5 of Finnish population). Urban and industrial areas cover approximately 15% of the basin area, which is very high proportion in Finnish scale. For the study area, we selected two waste water treatment plants (WWTP), a landfill site, river sites and sites from the river estuary as well as sites located close to the off-shore outlet of the treated waste water from WWTP Viikinmäki (800 000 inhabitants). In Viikinmäki WWTP, the annual average volume of treated waste water is 100 million m^3. The selected middle-sized WWTP Kalteva (40 000 inhabitants) is located 65 km north from the river mouth. From Kalteva the treated waste water (almost 4 million m^3 per year) is directed into river Vantaanjoki. River samples were taken in one upstream and several downstream sites from Kalteva. The third "point source" sampling site was a landfill, Metsä-Tuomela, where some WWTP sludge is composted among other processes. Its leachate is collected and treated in a small WWTP (about 0.001 m^3 per year) located in the landfill area. Pharmaceuticals were found in all studied matrices from the WWTP influents and effluents to river water and the Baltic Sea. Several active ingredients of pharmaceuticals were analysed first time in Finnish point sources and in the environment. The observed concentrations of selected compounds are presented in the poster with their daily loads from the WWTPs, land-fill site and river sites at the three sampling dates. The approximated annual loads are given with a rough uncertainty estimation. This study is one of the eight case studies carried out around the Baltic Sea area in a project "Clear waters from pharmaceuticals" (CWPharma) funded by EU's Interreg Baltic Sea Region Programme. The results will be utilized in a pharmaceutical loading model. Concentration data is used also in the ecological risk assessment of pharmaceuticals in the Baltic Sea Region.

WE075

The impact of human drug metabolism and disposition on the occurrence of pharmaceuticals in wastewaters - A case study on Finnish influent wastewater

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The ecotoxicological risks related to pharmaceutical emissions are an increasing concern socially, both locally and globally. With societal transformation (urbanization, population growth and ageing), pharmaceuticals are emitted into urban water cycles and into the environment at an accelerated pace. To be able to understand the full impact of pharmaceutical residues on the environment, it is important to identify and select the most risk-bearing compounds for environmental monitoring. Presently, the occurrence of pharmaceuticals in wastewaters is almost entirely assessed based on the original, therapeutic form of a pharmaceutical ingredient, while many of them undergo significant metabolism (molecular reaction) in humans. As the result of metabolism, many pharmaceuticals are excreted (in urine and faeces) in a completely different molecular form, which remains undetected if the analytical method is targeted solely for monitoring of the original form. However, the human metabolites may also be pharmacologically active and thus toxic to non-target organisms. The key question therefore is, how to choose the correct molecular form(s) of each pharmaceutical for environmental monitoring. In this study, we show how the existing drug metabolism databases (e.g., DrugBank) and scientific literature can be exploited to interpret the expected and unexpected observations on the occurrence of pharmaceuticals in wastewaters. Here, we examined the concentrations of selected pharmaceuticals (original, therapeutic form) in influent wastewater at four wastewater treatment plants (WWTPs) located in Southern Finland. The influent concentrations, coupled with information on wastewater flow, were used in calculating an estimated cumulative load of each substance to the WWTPs. The estimated loads were then further extrapolated to a national level based on national statistics on wastewater flow. The correlation between the estimated national cumulative load and national drug consumption based on annual sales statistics was then assessed case by case with a view to explain the conformities (e.g., high consumption, high load) and deviations from the correlation (high consumption, low load) with help of drug metabolism and disposition data.

WE076

The influence of microbial community and chemical properties on biodegradation rates in municipal wastewater

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Around 300 million tons of synthetic organic micropollutants (MPs) are being produced annually. Via industry and households, part of these MPs end up in the sewage and are transported to wastewater treatment plants (WWTPs). Removal rates vary between WWTPs and the driving factors behind that variation are likely related to the different microbial communities in WWTPs, but largely unknown. Next to the microbial metabolism, biodegradation of MPs is also influenced by physicochemical properties of the MP and WWTP conditions. Currently, the effect of MP properties on biodegradation can be fairly well described with quantitative structure activity relationships. However, effects of the biological system are often not included and a measure for microbial activity is lacking in models predicting primary biodegradation. Although the number of microorganisms is relatively constant in WWTPs, the active microbial community and biodegrading enzymes are not. Adaptation of microbial community occurs, depending on the composition of the wastewater. Here, we study the effect of micropollutant concentration on biodegradation in order to unravel the effect of MP properties and concentration on biodegradation by the microbial community. We aimed to describe the first biodegradation step with a Monod equation in addition to QSAR modelling, considering biodegradation as a second order reaction. We hypothesized we could predict biodegradation at low MP concentrations by means of QSAR modelling. With an increasing MP concentration, we expected biological processes to become more important because of substrate competition, limitation and adaptation of the microbial community. The selected MPs consisted of chemicals that were expected to undergo C-N cleavage and that show a variable biodegradation rate. Biodegradation rates were assessed in batch reactors and we observed microbial community change in 5 days, after spiking wastewater from a nitrifying reactor at a municipal WWTP (Groesbeek, the Netherlands). Carbamazepine, diclofenac, fluoxetine and acetaminophen were incubated at a concentration of ~ 1, 10 and 100 μg/L. Subsequently, sorption of the MPs onto sludge and the biodegradation rate were calculated. We observed microbial community change and transcript abundance of the ammonium oxidizing gene 'amoA' over time. On my poster, you can see how a concentration gradient of micropollutants affected the microbial community and corresponding biodegradation rate.

Ecological Impact and Management of Dumped Munition Sites (P)

WE077

Overview of the results of the monitoring campaigns for analysis of Baltic Sea sediment samples for sea-dumped chemical warfare agents

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After the Second World War, large amounts of chemical munitions containing chemical warfare agents (CWAs) were dumped into the Baltic Sea. In 2006-2018, Bornholm, Gotland deep, Skagerrak, and Gdansk deep dump sites were studied in many projects firstly in the EU FP6 project *Modelling of Ecological Risks Related to Sea-Dumped Chemical Weapons" (MERCW)*. This project was later amended by Baltic Sea Region Interreg Project *Chemical Munitions, Search and Assessment (CHEMSEA)*, NATO SPS project *Towards the Monitoring of Dumped Munition Threats" (MODUM)* and a joint EU project *Decision Aid for Marine Munitions" (DAIMON)*. The presence of various CWAs was studied. The results indicate a widespread contamination reaching far beyond the dumpsite boundaries, as CWA degradation products were detected in most of the sediment samples taken from and outside the dumpsites. The contamination is mostly related to arsenic containing compounds like Clark I/II, Adamsite, arsenic oil, and inorganic arsenic. Some samples also indicated the presence of sulfur mustard and its degradation products. Due to the presence of multiple, overlying sources of contaminants, the correlation between detected compounds and CWA concentrations is not always straightforward. However, overall results strongly suggest that the sea-dumped munitions are indeed leaking and the contamination has eventually reached the seafloor sediments. Overview of the analysis results and located hotspots are visualized in the geographical maps. The analysis data is also fully loaded into a Decision Support System (DSS), which has been produced in the DAIMON project and is available for authorities, providing risk assessment and decision aid related to operations in the contaminated areas.

WE078

Integrated biomarker analysis and assessment in cod (Gadus morhua) from dumpsites of chemical munitions in the Baltic Sea

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Dumping of chemical munitions and chemical warfare agents (CWA), mainly from German production, took place in the deep basins of the Baltic Sea after WW 2 by order of the allied forces, and it is estimated that between 30,000 and 50,000 t were disposed. In the framework of the CHEMSEA project (2011-2013), cod (*Gadus morhua*) from CWA dumpsites located in the Bornholm Basin, Gotland Deep and Gdansk Deep as well as from a munitions-free reference site outside the Gdansk Bay were studied for spatial patterns in biomarker responses. A battery of established biomarkers was analysed, reflecting exposure, early or chronic contaminant effects. These included enzymatic (acetylcholinesterase, glutathione S-transferase, glutathione reductase, catalase), cell and tissue (lysosomal membrane stability, lipofuscin accumulation, apoptosis, liver histopathology) as well as fitness and health biomarkers (condition index, diseases and parasites). For the integrated analysis of biomarker data obtained, a modified version of the Integrated Biomarker Response (IBR) approach was applied, introducing weighting factors for each biomarker as an additional component, emphasizing the significance of the response/effect for the host. The results reveal marked spatial differences in the IBR results, with the major CWA dumpsite (Bornholm Basin) showing the strongest and the reference site outside Gdansk Bay the lowest multi-biomarker response. For the assessment, also an integrated approach is suggested that either utilizes established or newly developed assessment criteria (BAC: background assessment criteria; EAC: environmental assessment criteria) for each biomarker, which are combined into an integrated multi-biomarker assessment based on individuals and sampling areas.

WE079

Toxic effects of chemical warfare agent mixtures on the mussel Mytilus trossulus in the Baltic Sea: a laboratory exposure study

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Approximately 50 000 tonnes of chemical weapons containing estimated 15 000 tonnes of chemical warfare agents (CWA) were dumped into the Baltic Sea after the World War II. Metal shells are corroding and contents leaking into the environment at an unknown rate posing a potential risk for the Baltic Sea ecosystem. In addition to mustard gas, arsenic based irritants Clark I and II (diphenylchloroarsine), Adamsite (10-chloro-5-hydrophenarsazine) and the tear gas α-chloroacetophenone (2-chloro-1-phenylethanone) were the most important CWAs dumped. Organo-arsenic compounds are considered as CWAs of major concern for biota; however, knowledge of their effects in marine organisms is scare. Degradation products of Adamsite are persistent and have great bioaccumulation potential. Moreover, Clark hydrolyses to diphenylarsenious acid that is known to be even more toxic than the parent compound. Degradation products of Adamsite and Clark have been detected in sediments and pore water in and outside of the major Bornholm dumpsite. In the present study, Baltic mussels (*Mytilus trossulus*) were exposed to different CWA mixtures containing low, medium and high concentrations of Clark I (1.25, 2.5, 5μg/L), Adamsite (2.5, 5, 10μg/L) and chloroacetophenone (5, 15.8, 50μg/L). The CWA mixtures were added based on the D-optimal design encompassing a treatment combination with all CWAs at low concentrations, combinations with one chemical at the low and all others at the high concentration as well as a triplicate centre point with all chemicals at medium concentrations. Mussels were exposed to differing mixtures of the three CWAs, solvent controls (50 μl/L methanol), and controls (no added chemicals) for 96 h. Each treatment consisted of 8 glass aquaria of 12 L in size containing 7 mussels (3.0 ± 0.25 cm). Water exchange and re-dosing of test compounds was conducted daily. Using a multi-biomarker approach the mussels were assessed thereafter for biological effects at different organisational levels ranging from geno- and cytotoxic effects to enzyme kinetics and immunological responses. The results showed that mussels bioaccumulate the oxidized forms of Clark I, Adamsite (DAox and DMox) and, to a certain extent, also chloroacetophenone into their tissues. Impairments including cytotoxic, immunotoxic and oxidative stress effects were visible in the test organisms even at the lowest test concentrations.

WE080

Toxicity of an oxidation product and a metabolite of the chemical warfare agent Clark I/II determined using the rainbow trout liver cell line RTL-W1

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After the first and second world wars, thousands of tonnes of chemical warfare agents (CWAs) were dumped in the Baltic Sea. Recent studies have found CWA-related arsenical compounds accumulated in fish tissues. The potential effects of dumped chemical munitions in Baltic Sea marine biota are still poorly understood. To link the measured chemicals in the fish to biological effects, cytotoxicity of diphenylarsenic acid (DPA[ox]) an oxidation product of the chemical warfare agent Clark I/II, was studied using the rainbow trout liver cell line RTL-W1. Additionally, toxicity of diphenylarsine glutathione conjugate (DPA-SG), which is a metabolite of DPA[ox], was also studied. Cytotoxicity of the compounds was evaluated using the Neutral Red retention test (NRR), showing a LC_{50} value of 294 mg/L for DPA[ox]and 1.32 mg/L for DPA-SG, indicating that the glutathione conjugate of DPA[ox]is more than two orders of magnitude toxic than the DPA[ox] itself. The kinetics and metabolism of DPA[ox]were also studied by incubating the RTL-W1 cells in media with 100 mg/L DPA[ox] and measuring the CWA-related phenylarsenic compound taken up by the cells by ultra-high performance liquid chromatography connected to high resolution mass spectrometry (UPHLC-HRMS). Also, DPA[ox]concentration in the cell media was measured in order to compare the fate of DPA[ox]in cell media to intracellular DPA[ox]concentrations after 30 min, 1 h, 2 h, 3 h and 4 h exposure. Toxicity of trinitrotoluene (TNT), a conventional explosive found in many dumped conventional munitions, was studied with the same method, and the LC_{50}

for this substance was found to be 61 mg/L. These studies will elucidate the biological effects of dumped conventional and chemical munitions, and help in assessing the environmental and health risks posed by their continued presence and deterioration in the sea bottoms.

WE081

Industrial scale destruction of old chemical ammunition of the Great War on the western Front The hundred-year-old forgotten contaminations

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During WWI, ammunition had been used on an unprecedented scale. When hostilities ceased, huge ammunition dumps remained. Unexploded ordnances were collected on former battlefields too. There was an urgent need to dispose million tons of hazardous ammunition. New methods for safely breaking down chemical shells were then developed.. After defusing, the rounds were emptied by perforation when the toxic contents were liquid, by steam or hot-water washing-out for TNT explosive shells and by open-burning for both chemical and explosive shells. These processes caused severe top soil contaminations especially on former burning-grounds of chemical shell. Recent research has been conducted on 9 burning-grounds in France and Belgium. To this day, no vegetation grows at some locations due to extreme high grade of heavy metal (Zn, Pb, Cd,…), ranging for Zn from 10 to 100 g/kg, chlorinated and /or brominated dioxin & furan (1 000 to 4 000 000 ng/kg), and arsenic (2 to 110 g/kg) when arsenical "*Blue Cross*" shells loaded with the sternutators diphenylchlorarsine and diphenylcyanoarsine were open-burned. Inorganic arsenical compounds are associated with organic by-products produced by the oxidation (diphenylarsinic acid) or thermal decomposition (triphenylarsine, As-PAH like phenylarsafluoren) of CLARKs. Brominated dioxin, energetic compounds (TNT, nitronaphtalin) and thianes (impurities of yperit) have been measured too. High resolution geomagnetic measurements had been conducted on a site where 1,5 million old chemical shells have been destroyed during the interwar period near Verdun, leading to severe top-soil contamination, crops and meat destructions and the sequestration of the ground. Open-detonation pits and systems of burning trenches have been recognized and carefully probed. Exposure to site-specific contaminants through the consumption of foodstuffs produced locally on the considered site was unlikely to be a health concern. However, as for inorganic arsenic, given the presence of highly contaminated zones, it was suggested that cereals should not be grown on certain spots. These site, Spincourt, is an example of the ignorance of soil contamination left behind by WWI in land-use planning. Further research is needed to assess these forgotten contaminations and sites and theirs related environmental risks.

WE082

Assessing antimony (III) speciation with the technique AGNES

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The core of small-arm bullets contains Sb in a proportion around 2 to 10% (in weight). The leaching of Sb from any kind of munition might be a source of pollution, with potential toxicity since Sb is non-essential for living organisms. However, there is a serious lack of quantitative knowledge about its speciation (not only between the redox states III and V, but also between different chemical species of the same redox state) [1]. In the case of Sb(III), hydrolysis is so prevailing that species such as $Sb(OH)_3$ and $Sb(OH)_2^+$ are dominant in solution even at acidic pH values. This information, about which are the complexed species carrying Sb(III), could be essential to perform ecotoxicological studies going beyond the correlation between total Sb(III) concentrations and biological effects, given that bioavailability is often associated to the concentrations and/or fluxes of very concrete species. To address this issue, analytical techniques should be designed or adapted. AGNES is an electroanalytical technique which has been successfully applied to the determination of free-cation concentrations for metals such as Zn, Cd, Pb, In and Sn in a variety of systems (dissolution of nanoparticles, seawater, river water, soil extracts, etc.) [2]. Sb can also be tackled with AGNES, as Sb(0) accumulates in the amalgam until fulfilment of equilibrium (according to Nernst law) for judiciously chosen potentials. A novel calibration strategy allows to correlate a deposition potential with a ratio between the concentration of Sb(0) and that of $Sb(OH)_3$ for a given pH. This strategy, together with optimized parameters (deposition times, deposition potentials, stripping currents, etc.) and speciation results will be presented. References: 1. Filella, M. *et al.*, Antimony in the environment: a review focused on natural waters II. Relevant solution chemistry. Earth. Sci. Rev. 2002, 59 (1-4), 265-285. 2. Companys, E., *et al.*, A review on electrochemical methods for trace metal speciation in environmental media. Current Opinion in Electrochemistry, 2017. 3(1): p. 144-162. The authors from the Universitat de Lleida gratefully acknowledge financial support from the Spanish Ministry MINECO (Project CTM2016-78798). PPV thanks Generalitat de Catalunya for a doctoral FI-AGAUR fellowship.

The Environment as a Reactor Determining Fate and Toxicity of Nanomaterials (P)

WE083

Toxicokinetics of Ag in the mealworm Tenebrio molitor exposed to different AgNP forms via food

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Application of sewage sludge onto agricultural land or the direct application of nanoagrochemicals can lead to exposure of soil and soil invertebrates to nanoparticles (NPs), with AgNPs being the most abundant NP type. To assess the risk of AgNPs, knowledge on the bioaccumulation of different forms of AgNPs in different terrestrial organisms is essential. The aim of this work was to determine the toxicokinetics of different AgNP forms in the mealworms *Tenebrio molitor*. Oat flakes, offered as food, were spiked with 100 mg Ag/kg dry food with 3-8 nm, 60 nm or 50 nm AgNPs, Ag_2S NPs (27 nm) and the ionic reference $AgNO_3$. Mealworms were kept on clean Lufa 2.2 soil and fed with the spiked food for a 21-day uptake phase and then transferred to clean soil and fed with clean food for a 21-day elimination phase. Three mealworms were collected at each sampling point (1, 3, 6, 9, 15, 21 days) for both phases. At each sampling time, animals and their molt (if they molted) were frozen individually (-20 ?C), dried, digested and analysed for Ag by graphite furnace Atomic Absorption Spectrophotometry. A one-compartment model with an inert fraction (F_i) was used to calculate the Ag uptake and elimination kinetics. The Ag toxicokinetics in mealworms exposed to different forms of silver via food showed no significant difference with uptake rate constant (k_1) values of 1.09, 1.74, 0.17, 3.12, and 0.25 g soil/g animal/day and elimination rate constant (k_2) values of 11.9, 14.5, 1.23, 19.5 and 2.42 day^{-1} for AgNPs 3-8 nm, AgNPs 60 nm, AgNPs 50 nm, Ag_2S NPs 27 nm and Ag^+, respectively. Moreover, Ag_2S NPs showed the lowest storage of Ag in an inert fraction (Fi = 0.01), while for AgNPs 3-8 nm, AgNPs 60 nm and Ag^+ the estimated Fi was similar at 0.7, 0.6 and 0.6, respectively, and for AgNPs 50 nm Fi was 0.3. The analysis of the mealworm molts showed that the amount of silver which can be eliminated by molting was quite high in case of Ag_2S NPs, with 71.8% of the body burden being found in the moult compared to those of AgNPs 3-8 nm, AgNPs 60 nm, AgNPs 50 nm and Ag^+, with 6.2, 4.1, 1.2 and 6.4 %, respectively. So, mealworms can excrete Ag by moulting, although the amount of Ag eliminated through moulting depends on the Ag form and its characteristics. The toxicokinetics in mealworms of different forms of Ag showed that different forms of Ag are handled in a different way by the organism.

WE084

The fate and effects of nano copper oxide to soil invertebrates

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Metallic nanomaterials are increasingly used in commercial and manufacturing applications world-wide. Concern over the fate of these engineered materials in the environment has led to efforts to improve our understanding of fate and effects, and how they differ from metal ionic counterparts. Copper (Cu) nanomaterials are used for a wide variety of applications due to its conductive and anti-fungal properties. Engineered nano Cu materials are expected to be released to the environment ending up in the soil compartment either directly, through the leaching of nano Cu treated materials (e.g. treated lumber) or, indirectly through the application of contaminated biosolids to agricultural soils. The effects of nano copper oxide (nCuO) on earthworms (*Eisenia andrei*) and springtails (*Folsomia candida*) were evaluated through reproduction toxicity and bioaccumulation tests. A field collected agricultural soil was spiked either directly or through biosolid amendment with 40nm nCuO or, with $CuSO_4$. The fate of the nanomaterial was examined through measurements of test soils for: total Cu (using ICP-MS), Cu^{2+} ionic activity (using an ion selective electrode) and particulate Cu (using ICP-MS in single-particle mode, and TEM). By understanding the fate in the soils, we can better understand what form of Cu test organisms are exposed to and, hence the most likely cause for any observed effects.

WE085

Multiple sewage sludge application into soil - Fate and effect of nanosilver in an outdoor lysimeter study over 5 years

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Nanomaterials (NM) will enter the environment via diverse pathways. Sewage sludge is repeatedly applied as fertilizer on farmland due to its high nutrient content. This may lead to a significant increase of NMs in soil over years. Our aim was to investigate the accumulation, plant uptake and additive effects due to multiple sewage sludge applications into soil for two years after a second application of Ag-NM via sewage sludge. In 2014, two concentrations of 1.8 and 7.0 mg Ag-NM/kg dry matter (dm) soil were applied via sewage sludge into the top 20 cm of lysimeter soil. The sludge was mixed into the top 20 cm to simulate ploughing. Over 3 years, the lysimeters were cultivated according to good agricultural practice. The effect on soil microorganisms was investigated as well as the fate of the NM in soil and the potential uptake into wheat, canola and barley (Schlich et al., 2017). No detectable horizontal displacement in combination with very low remobilization for both tested NM over 3 years was found indicating that the sludge applied NM remained nearly immobile in the pathway between soils and leachate. However, Ag uptake in the roots of wheat, canola and barley indicated that the chemical conditions in the rhizosphere induce Ag-NM remobilization from the incorporated sewage sludge even after three harvesting cycles. A constant inhibition of the soil microflora (ammonium oxidizing bacteria and substrate-induced respiration) was observed over 3 years at 7.0 mg/kg dm soil, while there was no effect at the lower Ag-NM concentration. Then, the experiment was continued for further 2 years. In accordance to the German Sewage Sludge Ordinance 5 tons of dry matter sludge can be applied on a hectare in 3 years. Therefore, after 3 years a new application of Ag-NM onto the lysimeter with an Ag-NM content of 1.8 mg/kg dm soil via sewage sludge was performed aiming in an Ag-NM content of 7.0 mg/kg dm soil. Only uncontaminated sludge was applied on the lysimeter, which already had an Ag-NM content of 7.0 mg/kg and onto the control lysimeter. After the application in April 2108, wheat was cultivated followed by bare fallow after the wheat harvest in October 2018. In regular intervals, the effect of the Ag-NM on soil microorganism was observed. In addition, the wheat plants were investigated for their Ag content in roots, shoots and ears. Once per month the leachate was collected and the Ag concentration was determined. Results until April 2019 will be presented.

WE086

Sorption of (nano)formulations difenoconazole in soil

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Nanopesticides represent an emerging technological development that aims to improve the efficacy of existing pesticide active ingredients and/or to enhance their environmental safety profiles. Due to small size (nanometer size range), nanoformulations are expect to have significant impacts on the environmental fate of the active ingredient (a.i.). Sorption is one of the major processes that determine the fate, effects, efficacy and ecological risks of pesticides in terrestrial and aquatic environments process. The aim of this study was to evaluate the extent to which different formulations may affect the fate of difenoconazole in soil. Soil sorption of difenoconazole was measured for i) pure active ingredient, ii) commercially available formulation (particle size of 174 ± 7 nm), iii) formulation with polymer (565 ± 27nm), iv) formulation with conventional polymer (959 ± 55 nm). Standard batch equilibrium method (OECD no. 106, 2000) was applied to determine the fate properties of formulations in three agricultural soils. The experiment showed that there were differences upon comparison of the results obtained for the polymer (565 ± 27nm) and commercial formulation with that of the pure a.i. Pesticide formulation with polymer (565 ± 27nm) and commercial formulation yielded two fold higher soil distribution coefficients (Kd values) in all three soils relative to that of the pure a.i. Laboratory experiments showed that the nanoformulation could increase the measured sorption coefficients.

WE087

Investigations on release of nanomaterials from waste and sewage sludge incineration residues during deposition and agricultural use-comparison of pilot scale simulation and laboratory tests

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Engineered nanoparticles (ENPs) are used in consumer products such as textiles or paints. Thus, it can be expected that a considerable fraction will enter the waste stream, which means in most cases incineration and deposition of the incineration slag in landfills. Most of the sewage sludge in Germany is also incinerated, but the incineration ashes are frequently used in agriculture as fertilizer. Investigations with CeO_2-ENPs by Walser et al [1] showed, that nanoparticles are possibly not permanently bound to incineration residues and may become released. The aim of the present project was therefore to investigate the possible release of TiO_2-ENPs in two different scenarios: (i) pilot scale landfill simulation of ENP-amended domestic waste after incineration and (ii) simulation of agricultural use of ashes from ENP-amended sewage sludge after application to soil. For landfill simulation a special reactor was used which allows to set up conditions present in a real landfill. For agricultural use, the same system was used but for time lapsed seasonal simulation by repeated freezing / thawing of the material. Both simulations had a scale of about 1.5 m^3 and were periodically applied with artificial rain in order to obtain leachates. The leachates were analysed for total titanium-concentration (ICP-OES after digestion) and for the particle concentrations and size distributions by means of single particle ICP-MS (spICP-MS). For both scenarios, reference simulations without spiking of TiO_2-nanoparticles were run simultaneously. For the domestic waste landfill simulation, no significant release of titanium-containing nanoparticles was observed compared to the reference simulation, indicating that the added nanomaterials are strongly bound to the incineration slag. In contrast, in the simulation of agricultural use of sewage sludge ashes the observed release of nanoparticles in comparison to the control simulation was significant and even higher than could be expected from the amount of TiO_2 spiked. The results from the simulation experiments could be reproduced with the same materials in standard laboratory soil elution experiments according to DIN 19528. [1] Walser, T. et al, *Nature Nanotechnology*, **2012**, 7, 520-524

WE088

Processes and transformations of engineered TiO2 nanoparticles in aquatic and terrestrial natural environments

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During the production of NPs TiO2 by the sulfate process (necessary to obtain the anatase form) releases occur in the last stages of manufacture and more particularly during the steps of washing, neutralization, filtration, atomization / calcination and grinding / sieving titanium dioxide. The industrial process generates effluents that may contain TiO2 NPs that are sometimes released directly into natural water courses, thus affecting water and sediments. The soils can be impacted by particles from emissions and redeposited after transport by wind. The fate of these nanoparticles is strongly related to their intrinsic characteristics. Once deposited by winds and precipitation or emitted directly to soils and water, NPs TiO2 form aggregates: their important surface area induces the presence of many reactive sites. This characteristic leads to an instability of these compounds which generates aggregation processes: homoaggregation, containing only agglomerates of NPs TiO2 or heteroaggregation, containing agglomerates of NPs TiO2 and particles of the natural environment. These processes are governed by different parameters of the physical environment where NPs TiO2 are found (pH, ionic strength, percentage of organic matter, presence of other chemical compounds) and parameters specific to nanoparticles (size, zeta potential, concentration). It is therefore essential to acquire information and better knowledge on the behavior of NPs TiO2 in the environment in order to evaluate their fate in the different environmental compartments. For this purpose, it requires firstly to detect and quantify TiO_2 NPs in water, soil and sediment near a production site of TiO_2 NPs in Vieux-Thann (68) and secondly to determine total titanium concentrations in soil, water and sediment samples by ICP-AES. The results show that water and sediment samples located near and downstream of the site production are 2,5 to 20 times more concentrated than the upstream point (used as a reference) which imply an impact coming from the production site. However, soil samples are only 1 to 1.5 times more concentrated than the geochemical background.

WE089

Impacts of pristine AgNPs and Ag2S-NPs on the growth and reproductive success of the pond snail Physa acuta

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Due to its wide use in antibacterial products, silver nanoparticles (AgNPs) end up in wastewater treatment plants (WWTPs) being discharged into the aquatic environment. In WWTPs, AgNPs undergo physical-chemical transformations, which modify their properties. It is crucial to consider such transformations when assessing their impacts to aquatic organisms. The freshwater snail *Physa acuta* was used to assess the potential toxic effects of pristine AgNPs and Ag_2S-NP, which simulate the transformation underwent in WWTPs, and to compare their toxicity. Chronic tests were performed to assess effects on survival, shell length, reproductive success (expressed as cumulative number of egg clutches and eggs per individual) and intrinsic rate of population increase. Both NPs caused mortality no higher than 20% at the tested concentrations. Exposure to pristine AgNPs caused a decrease in the shell length at the highest tested concentration (600 μg/L) while Ag_2S-NPs caused an increase in shell length at 300 μg/L. Pristine AgNPs caused a decrease in the clutch production and cumulative number of egg per individual with increasing concentrations, while Ag_2S-NPs had no effects on the cumulative number of eggs per individual and a beneficial effect on clutch production. Exposure to pristine AgNPs resulted in a decrease in the intrinsic rate of population growth at the highest tested concentrations (300 and 600 μg/L). Snails exposed to Ag_2S-NPs showed an increase in the intrinsic rate of population growth only at 37.5 μg/L. The frequency of egg abnormalities, used as an additional endpoint, was more responsive to both NPs than other endpoints such as cumulative number of egg clutch per snail. Polyembryony and eggs with atrophied albumen were the most frequent egg abnormalities. These type of egg abnormalities can provide additional information about physiological pathways disturbed by different Ag particulated forms. Besides, our results suggest that egg abnormalities are a more sensitive endpoint when compared to the classical

reproductive endpoints commonly assessed in toxicity tests with aquatic snails.
Keywords: nanomaterial, egg quality, hermaphrodite snail

WE090
Multiple effects of elevated temperature and acidification on accumulative dynamics of copper nanoparticles in freshwater fishes
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The potential risk to freshwater ecosystems posed not only by nanomaterial contaminant but also by temperature and acidification. To date, there is a lack of knowledge related to combined effects of relevant environmental change and copper nanoparticles (CuNPs)in freshwater ecosystems and their potential impact on freshwater organisms. This study examined effects of increased temperature and acidification on accumulation and depuration of CuNP in embryo and larvae of medaka (*Oryzias latipes*) and larvae tilapia (*Oreochromis niloticus*). Our findings indicated that high acidification appeared to be a major driver in increasing CuNPs accumulation of medaka embryo, whereas increased temperature had antagonistic effect against acidification on CuNP accumulation. However, there was no difference of Cu accumulation on medaka larvae among conditions of environmental changes in both uptake and depuration exposures. For tilapia, we also found slowly decreased the Cu depuration with high temperature after 1-d depuration exposure. The conditions of moderate acidification with moderate temperature as well as high acidification with high temperature slowly decreased Cu accumulation of tilapia, compared to control condition. It revealed the additive effects of elevated temperature and acidification on Cu depuration of tilapia. Differential species and life stages displayed varying level of CuNPs accumulation in response to the combined temperature and acidification. Our study demonstrated that the combined environmental changes may not lead to adverse effect of CuNPs on aquatic species. We conclude that acidification is a major driver than temperature in increasing the accumulation of metal nanoparticle in freshwater fish.

WE091
Toxicokinetics of pristine and aged silver nanoparticles in freshwater benthic organisms: the role of exposure route
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Nanomaterials (NMs) can suffer several physico-chemical changes during their life-cycle. When released into the environment, NMs may reach biological receptors in unpredictable forms, which can determine their fate through different uptake routes. Moreover, organisms can also act as bio-reactors and induce changes on NMs during internal processing. In order to understand and assess NM bioavailability, ways of uptake and elimination and biological fate, it is necessary to conduct toxicokinetic modelling studies. In freshwater systems, benthic organisms can be exposed to NMs through water and sediment and therefore different routes and rates of NMs are expected. The present study aimed at determining the toxicokinetics of silver nanoparticles (Ag-NPs), in their pristine and aged form, through different exposure routes, in the freshwater benthic organisms *Physa acuta* (pond snail) and *Dugesia tigrina* (planarian). Bioaccumulation assays were performed with three pristine Ag-NPs of different sizes (3-8 nm, 50 nm and 60 nm), a simulating aged form (Ag_2S-NP, 27 nm), and $AgNO_3$ as ionic control. Exposures were through 1) contaminated aqueous medium; 2) contaminated aqueous medium and clean sediment; 3) contaminated sediment and clean aqueous medium. In all experiments, organisms were exposed to contaminated medium during the uptake phase and then transferred to clean medium for the elimination phase. Organisms were sampled throughout the tests and total body Ag concentrations were analysed by graphite furnace atomic absorption spectrometry. Toxicokinetics of Ag were derived by one-compartment models. Results revealed higher uptake rates and bioaccumulation factors (BAF) for the snails, while planarians presented, in general, low uptake rate constants (k1) and low BAFs. Planarians showed the highest uptake rate for the aged form (although with lower k1 than the snails) in the exposure with contaminated water and clean sediment. Snails exhibited similar uptake rates between exposures through contaminated aqueous medium only (no sediment) and contaminated aqueous medium with clean sediment, being higher for ionic Ag and 60 nm Ag-NP. However, for Ag_2S-NPs, the snails showed one of the highest k1 values in the exposure with contaminated aqueous medium (no sediment), while for the exposure with contaminated aqueous medium and clean sediment they presented one of the lowest k1. This suggests that exposure routes may have a determining role on the uptake rates of these NPs.

WE092
Zinc oxide nanomaterials toxicity to Daphnia magna. Effect of particle coatings on immobilization, reproduction and body size.
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Zinc oxide nanomaterials (ZnO NMs) have remarkable optical, physical, and antimicrobial properties, which result in their use in a wide range of applications such as biosensors, cosmetics, drug-delivery systems, sunscreens, biocides. In general, the purposes of surface coating on nanomaterials (NMs) are: extending their applications, changing the properties, or reduce toxicity (safe-by-design). In this study, the ecotoxicity of triethoxycaprylsilane coated ZnO NMs (152 nm) and uncoated ZnO NMs (70-90 nm), obtained from the Joint Research Centre of the European Commission, were studied using *Daphnia magna* as model species. The Acute Immobilisation Test (OECD 202) and the Reproduction Test (OECD 211) were employed in this study. The parent organisms were cultivated with 16 hours light/ 8 hours dark lighting cycle and fed with alga *Chlorella vulgaris* daily. Neonates aged less than 24 hours were used in the acute experiments. For the preparation of the ZnO NMs suspensions, the protocol from the Nanogenotox project was followed and the initial concentration of ZnO NMs was 2.56 mg/mL. The results indicate that uncoated ZnO NMs and coated ZnO NMs all revealed acute toxicity toward *D. magna*. The 48-hours EC50 values for uncoated and coated ZnO NMs were in the similar scale, around 2.3 to 3.5 mg/L. However, in the chronic reproduction test, uncoated ZnO NMs showed higher mortality than coated ZnO NMs. With the concentrations ranging 180, 300, 500 to 840 µg/L, the parent *D. magna* mortality was 10%, 10%, 30%, 70% in the coated ZnO NMs group, respectively. In contrast, it was 60%, 50%, 90%, 100%, respectively, in the uncoated ZnO NMs group. Furthermore, in the coated ZnO NMs group, neonates per parent daphnia and the body size in concentrations 180, 300, 500 and 840 µg/L were significantly lower compared to the control group; there was also a clear trend showing that uncoated ZnO NMs exposures led to a decreasing number of neonates per parent daphnia. Based on the present data, it is suggested that coated ZnO NMs are less toxic than uncoated ZnO NMs toward *D. magna* in longer-term exposures. It is suggested that further studies, including more replicates and other aquatic species, should be carried out. The final goal of this study is to continue to support nanosafety, working closely with industry, which will benefit both environment and human health.

WE093
The effects of ageing on fate and toxicity of coated silver nanoparticles to freshwater alga Raphidocelis subcapitata
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The fate and transformation of AgNPs in the aquatic environment together with their impacts on organisms remain a research area requiring further study, given the increasing release of AgNPs into the environment. In this study, effects of ageing on the fate and behaviour of coated AgNPs; tyrosine (T-AgNP), epigallocatechin-gallate (E-AgNP) and curcumin (C-AgNP), in the algae medium and on the sub-lethal effects in the freshwater alga, *Raphidocelis subcapitata* were studied. The stability of the AgNPs was evaluated by monitoring surface plasmon resonance, zeta average hydrodynamic diameter, zeta potential and dissolution of the NPs over 32 days. Results indicated that the transformation of all three types of AgNPs occurred during the incubation, while coating-specific effects were observed. The presence of the three types of AgNPs resulted in increased ROS formation in algae compared with the control ($p < 0.05$) while aged T-AgNPs and C-AgNPs induced excessive ROS generation compared with the fresh counterparts. Increased ROS levels caused increased lipid peroxidation in the treatment groups exposed to both fresh and aged NPs while the TBARS levels were higher in algae exposed to aged AgNPs. The observed increase in CAT activity of algal cells was attributed to early stress responses induced by excessive intracellular ROS generation while the CAT levels were higher in the aged NP treatment groups in line with the increased ROS levels. It can be concluded that AgNPs have a negative effect on aquatic algae, as manifested by the increased ROS levels and lipid peroxidation while antioxidant enzymes such as CAT are activated to neutralize the oxidative damage. Overall, the results suggest that the ageing and coating of AgNPs have major impacts on AgNP transformation in media and their effects to algae.

WE094
Bioaccumulation of radiolabelled weathered multi-walled carbon nanotubes in various aquatic organisms: water and dietary exposure
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Carbon-based nanomaterials like carbon nanotubes (CNT) are incorporated in various consumer plastic products. During production processes and after usage they finally enter the environment. Due to environmental complexity, fate of CNT might be altered by various biotic and abiotic influences. Herein, radiolabelled multi-walled carbon nanotubes irradiated by simulated sunlight (65 W/m^2; 3 month, ^{14}C-wMWCNT) were used in bioaccumulation studies considering bioconcentration (water exposure) and biomagnification (dietary exposure). The primary producers *P. subcapitata* and *C. reinhardtii* and the primary consumer

Daphnia magna were chosen as testorganisms. In tests on bioaccumulation of wMWCNT (100 μg/L) in green algae the calculated log BCF for *C. reinhardtii* and *P. subcapitata* after 24 h was 4.1 and 3.0, respectively. The higher bioconcentration of one magnitude in *C. reinhardtii* can be explained by the different shape or size of algae cells and the difference in exudate components. In a second experiment, CNT-loaded algae (72 h) were fed to *D. magna* for dietary exposure and results were compared to water exposure (100 μg wMWCNT/L). In water exposure scenario, maximum uptake was reached after 19 h with 7.1 ± 1.5 μg wMWCNT/mg dw. In the following 53 h, a decrease in body burden was observed. The maximum amount of CNT uptake during dietary exposure was already reached after 2 h. Data revealed a 100-fold lower uptake of 0.07 ± 0.01 μg wMWCNT/ mg dw in comparison to water exposure. The calculated log BCF for water exposure was 5.2. At first sight this indicates a really high bioaccumulation potential for CNT in water fleas, but also gut accumulated CNT were included in this amount. To determine elimination a second test was constructed, i.e., the influence of presence and absence of food was examined. After an uptake phase of 24 h, daphnids were placed into fresh medium and elimination over 48 h was measured. It could be observed, that in presence of food source elimination processes were significantly faster than without food. Results show, that the amount of CNT taken up is eliminated almost completely after a certain time which indicates, that MWCNT appear to be substances with low or no concern for bioaccumulation. **Acknowledgements** The work is supported by the European Project NANO-Transfer that receives funding from the Bundesministerium für Bildung und Forschung (BMBF) under agreement with the FP7 ERA-NET SIINN.

WE095

New challenges to evaluate potential risk of nanoscale copper phthalocyanine in the aquatic environment

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Nanoscale copper phthalocyanine (n-CuPc) is a pigment widely used in paints to enhance automobile coatings and make colors transparent and more appealing to users. This application implies high probability of end-of-life release during recycling and/or after disposal, which may lead to environmental exposure and risks. However, assessing potential environmental and human health risks of n-CuPc is very challenging as new science at the interface of biological system and nanomaterials is emerging only now and sufficient and reliable risk data are often lacking. During the product life cycle, interaction of n-CuPc with the environment changes significantly their physical and chemical properties thus affecting exposure and risk. Even though (eco)toxicity of n-CuPc has been evaluated extensively, the testing was conducted under assumption that material is stable in the environment and no nano-specific transformation properties were addressed in the test design. Therefore, the old testing protocols on n-CuPc may not result in a mechanistic understanding of the induced (eco)toxicity in nano-form. To understand the knowledge gaps of n-CuPc behaviour in the aquatic environment, the biodynamic of n-CuPc in *Daphnia magna* was conducted in our laboratory. The age of 5 days' *D. magna* was exposed in M7 medium containing n-CuPc (0.1mg/L). The *D. magna* was sampled at 1 h, 4 hrs, 8 hrs, 16 hrs and 24hrs to analyze the bioaccumulation of n-CuPc by ICP-MS. After 24 h, *D. magna* was transferred to M7 media for n-CuPc depuration. *D. magna* was sampled at 1 h, 4 hrs, and 24 hrs to analyze n-CuO amount in *D. magna*. The biokinetics results show increased uptake of n-CuPc with the exposure time within first 16 hrs and saturation at 24 hrs. n-CuPc can not be depurated in *D. magna* in 24 hrs. Our results are not consistent with the previous test data which showed no n-CuPc bioaccumulation in environment. The difference in the results may be due to the different methods of n-CuPc suspension preparation in media compare to the old protocols and the n-CuPc specific physical-chemical properties which traditional testing protocols were not detecting. Our study provides evidence that n-CuPc show complete different biokinetics as compared to the testing protocols used before. A new protocol for re-evaluation of potential exposure and hazard of n-CuPc in the aquatic environment is urgently needed to ensure safe use of n-CuPc in growing market applications.

WE096

Toxicity mitigation by N-acetylcysteine and photo-induced toxicity of zinc oxide nanoparticles on a bacterivor nematode: Panagrellus redivivus

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Nanoparticles have new physical and chemical properties as a result of their alteration in size, therefore they have to be particularly well characterized with several analytical techniques and with extensive biological research. Toxicity of ZnO particles (with 15 and 140 nm average particle size) to *Panagrellus redivivus* was examined under dark condition (in thermostat) versus under simulated solar UV radiation (in Plant Growth Chamber). N-acetylcysteine was used in toxicity mitigation assay to elucidate the importance of dissolved zinc ions as well as phototoxicity in ZnO toxicity. ROS generation and particle dissolution were also measured under both irradiation conditions. Both ZnO particles toxicity increased under solar UV radiation. Toxicity mitigation by N-acetylcysteine under dark and solar UV radiation conditions proves to clarify the role of particle dissolution and ROS generation in nanoparticles toxicity. After the mitigation of the toxic effect of dissolution and phototoxicity, there was still mortality in the higher concentrations of the 15 nm ZnO particles. We may conclude the nanorelevant toxic properties, like increased surface area, particle size or morphology were also playing a role in toxicity mechanism.

WE097

The ecotoxicological impact of titanium dioxide nanoparticles on the fish kairomone induced anti-predator defence in Daphnia magna

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In the last decade, the production volume of manufactured nanomaterials (MNMs) has dramatically increased due to their widely use in common products. Because of the bright white pigment, titanium dioxide nanoparticles (TiO_2NPs) are one of the most industrially used MNMs and are applied in daily items such as sunscreens, toothpaste, food and also in paints or coatings. The ecotoxicity of TiO_2NPs to the freshwater organism *Daphnia magna* is well investigated. The interaction between NP-exposure and one important mechanism of water fleas, the anti-predator defence, however, has not been studied yet. It is known that daphnia are able to produce offspring with longer spines or characteristic helmets as a phenotypic response to kairomones released from fish. The resulting increase in body size allows the daphnia to defend themselves against predators in their natural environment. As a key species in the food chain of the aquatic environment, daphnia are of great important for fresh water systems. Their ability to grow adequate defensive structures is, therefore, necessary to prevent an ecological imbalance in the freshwater environment. In this study, we investigated the ecotoxicological impact of TiO_2NPs (NM-105) on the anti-predator defence response in daphnia by chronically exposing *Daphnia magna* to fish kairomones from the tropical freshwater fish *Danio rerio* in combination with different nanoparticle concentrations. Following the OECD guideline No. 211, the experiment was performed over 21 days with a daily water change to ensure a high concentration of fish kairomones. As endpoints, we measured the body length and the tail spine length after each moult of the test animals and juveniles and counted the number of offspring.

WE098

Bioavailability and Bioaccumulation of Silver Nanoparticles in the Rainbow Trout Oncorhynchus mykiss

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Due to the increasing use of silver nanoparticles (AgNPs) in the production of various products, the risk of AgNPs being released into the environment rises. Even though AgNPs are mostly retained within wastewater treatment plants (WWTP), a small fraction of predominantly transformed nanoparticles enters the aquatic environment. Besides the numerous studies on ecotoxicological effects, information on the transfer, bioavailability and bioaccumulation of AgNPs in the aquatic food chain is essential for risk assessment. The aim of this study was to investigate bioavailability and bioaccumulation of waterborne and dietary AgNPs in the rainbow trout *Oncorhynchus mykiss*. Several model WWTPs were conducted according to OECD Guideline 303A. The effluents containing AgNPs (present or supplemented manually) were compared to pristine AgNPs and used to perform a bioconcentration study with *O. mykiss* according to OECD Guideline 305. Fresh zooplankton was collected in a fishpond in southern Germany and enriched with pristine AgNP. The loaded plankton was filtered, frozen, freeze-dried and processed into agar pellets, which can be eaten by fish. The resulting fish food was stable and displayed a homogenous AgNP distribution. The agar pellets were fed to juvenile *O. mykiss* in a biomagnification study according to OECD Guideline 305. Tissue concentrations during uptake and elimination in *O. mykiss* exposed to AgNPs via water or food were investigated by quantitative inductively coupled plasma mass spectrometry (ICP-MS). Enriched plankton samples were analysed by high-resolution transmission electron microscopy (TEM) with energy dispersive X-ray (EDX). For waterborne AgNPs present in or supplemented to WWTP effluents no significant uptake into the carcass of the test organisms could be detected. However, exposure to pristine AgNPs was leading to the accumulation of silver in fish tissues presumably driven by the uptake of silver ions released by the AgNPs. Biomagnification studies showed that pronounced silver concentrations could be found in the digestive tract. However, only a limited biomagnification of silver could be detected.

WE099

Tissue distribution of radiolabeled 110mAg nanoparticles in fish

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This work presents the first complete tissue distributions of dissolved (Ag_I) and silver nanoparticles (20 nm, Ag_0NPs_{20}) in fish (Arctic charr, *Salvelinus alpinus*). The distributions are provided for fish exposed to three different treatments including intravenous (IV), dietary and waterborne. Quantitative whole-body autoradiography (QWBA) analyses obtained on high-resolution images reveal distinct silver distribution patterns according to the treatments and the forms. The IV exposures showed that $AgNPs_{20}$were mainly located in bile and kidney after 8 d, while $Ag_I$was distributed through the whole body and reached particular tissues such as bones, eyes, skin, liver, spleen, kidney, and intestine. The Ag_0NPs_{20}distribution with the dietary exposures suggests some dissolution occurred within fish organs. We suggest that dissolved silver (i.e. from $Ag_I$and Ag_0NPs_{20}) could precipitate as chloride, sulfide or selenide and be incorporated in bones during the growth. As such, it is difficult to state if silver cross biological barriers in a nanoparticular form. Finally, the waterborne exposures revealed that the gills can capture Ag_0NPs_{20}in small quantity, implying that the stability of Ag_0NPs_{20}in water is critical.

WE100
Histopathological effects of silver ions and nanoparticles on zebrafish (Danio rerio) spleen and liver morphology during short time toxicity test
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Despite the fact, that nanotechnology is rapidly growing branch of industry, the toxicity of nanoxenobiotics has not been well understood yet. The aim of the study was to investigate influence of nanoparticles and silver ions on the morphology of zebrafish (*Danio rerio*) spleen and liver. Fish at the age of 5 months were exposed for a week to aqueous solutions of silver nanoparticles at concentrations of 0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 mg/L (AgNP groups) or aqueous solutions of silver ions at concentrations of 0.01 and 0.05 mg/L (Ag + groups). In the control group fish were kept in water without the addition of xenobiotics. All the animals were fed *Artemia salina* and commercial *ad libitum*. On the last day of the experiment, the fish were anaesthetized in MS222, fixed in glutaraldehyde to assess the ultrastructural changes in hepatocytes or in Bouin's solution and then subjected to a standard histological procedure. Obtained histological slides were stained with the hematoxylin/eosin methods to analyze liver and spleen morphology, AB/PAS to analyze deposition of lipofuscin in macrophages, and Perl to assess the deposition of hemosiderin in macrophages. On the last day of the experiment, survival rate was close to 100% in the all groups, except Ag+ 0.05mg/L. Microscopic analysis did not show any histopathological changes in liver and spleen fish from the control group. The area occupied by the lipid vacuoles in the cytoplasm of hepatocytes in liver was higher with increasing concentrations of studied nanoparticles and silver ions. In liver macrophages were not observed. In the spleen parenchyma fish from the control group no deposition of macrophages was found. In the groups exposed to the higher concentration of tested xenobiotics, the area occupied by macrophages forming the melanomacrophage centers in the spleen increase. The largest area of melanomacrophage centers (statistically significant) were found in the spleens of the fish exposed to AgNP 2.0 mg/L. In addition, intensive deposition of lipofuscin and hemosiderin was observed in melanomacrophage centers fish from the Ag + 0.05mg/L and AgNP 2.0 mg/L groups. The obtained results indicate that the increase concentration of xenobiotics may be affected on disturbance of homeostasis, activation of the immune system and deposition of lipofuscin (indicator of fatty acids peroxidation) and hemosiderin (reservoir of iron ions) in macrophages.

WE101
Toxicity evaluation and antioxidant activity of caffeic acid-loaded mesoporous silica nanoparticles
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The nanoencapsulation is an excellent strategy to increase the solubility, stability and bioavailability of antioxidants, such as caffeic acid (CA). Therefore, the aim of this work was to synthesize and evaluate the effect of mesoporous silica nanoparticles (MSiNP) and MSiNP coated with polyvinyl alcohol (MSiNP-PVA) loaded with CA on the microalgae *Raphidocelis subcapitata* growth and on Raw 264.7 cells viability, as well as to evaluate the antioxidant activity of CA released from nanoparticles. MSiNP were synthesized using tetraethyl orthosilicate (TEOS) as precursor, N-cetyl-trimethylammonium bromide (CTAB) as porous template and NH_3as catalytic agent. Coating of MSiNP was made using a solution of PVA (60 000 5%, w/v). For algae assay, stock solutions of MSiNP were prepared in algae culture medium and added to algae inoculum into microplate and maintained under photoperiod conditions during 72h.The cytotoxicity of the samples was evaluated by comparing the viability of non-exposed Raw 264.7 cells with exposed cells to different concentrations during 48h using AlamarBlue method. The antioxidant activity of CA released from nanoparticles was evaluated by nitric oxide (NO) and (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical assays. MSiNP-PVA reduced the algae growth in comparison to the control group. However, no toxic effects were observed when Raw 264.7 cells were exposed to nanoparticles. The NO scavenging activity of CA released from MSiNP was higher than that released from MSiNP-PVA at highest concentrations. However, in ABTS assay, no differences were observed in the antioxidant effect of CA between the results with MSiNP and MSiNP-PVA and the nanoparticles increased the scavenging capacity of CA with concentration. Data showed that the antioxidant activity of CA released was not affected by nanoencapsulation at least in NO radical assay. In general, MSiNP and MSiNP-PVA are biocompatible materials and their effect can vary depending on the test species.

WE102
Investigations on the uptake and fate of nanostructural polystyrene in the fresh water mussel Corbicula fluminea
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Polystyrene is one of the mostly used type of plastic and could enter the environment by direct release from industrial products during their usage as well as by their disposal. In the environment, those macro- and microplastics could undergo weathering through biological degradation, mechanical erosion and UV radiation and could thus be transformed into nanoplastic particles. Most nanoparticles (NPs) tend to sediment in water and are supposed to be primarily taken up by benthic species in aquatic ecosystems. Different studies have shown that mussels are able to ingest and to incorporate NPs suspended in water. By this the NPs may bioaccumulate in this organisms and thereby enter the food chain at a very low trophic level leading to potential further biomagnification. The filter feeding freshwater bivalve *Corbicula fluminea* was used to investigate the uptake of nanostructural polystyrene (nPS) from the water. By the use of fluorescent nPS and fluorescence microscopic analysis we were able to investigate the uptake, localisation and elimination of the nPS in the soft body of *C. fluminea* after different exposure and depuration times. During the exposure period the nPS induced a high filtration activity resulting in a strong production of (pseudo-)feaces, whereby the feaces showed a strong fluorescence intensity. Also the soft tissue of the mussel showed a high strength of fluorescence after 24 hours of exposition with different intensities for the different compartments like the mantle, the foot, adductor muscles and the viscera (including the gills and the digestive system). The results indicate that the particles are ingested, are temporarily adsorped to the tissue surfaces, finally pass the digestive system without being incorporated into the organisms tissues and are obviously not bioavailable for mussels. A rapid and effective elimination of the nPS was observed after 24 hours of depuration.

WE103
Condition dependent aging of nanosized titanium dioxide influences copper toxicity towards the water flea Daphnia magna
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Nanosized titanium dioxide ($nTiO_2$) has wide applications in consumer products and is inevitably released into the environment including freshwater bodies. During their aquatic life cycle, $nTiO_2$ may interact with natural organic matter (NOM) affecting ecotoxicological consequences of co-occurring chemical stressors such as heavy metals. The ecotoxicological consequences of these interactions are influenced by various factors including aging duration of $nTiO_2$ in the environment complicating a valid risk prediction. The present study aimed at assessing the influence of $nTiO_2$-aging conditions on the toxicity of copper (Cu) for various aging durations under freshwater conditions using a factorial test design. The test design crossed two $nTiO_2$ levels (0.56 versus 2.57 mg/L) with two levels of NOM (0 versus 8 mg/L TOC) and seven nominal Cu concentrations (ranging from: 0 – 1536 µg/L) for a duration of 0, 1, 3 and 6 days, while two types of aging conditions were realised. The Type 1 aging condition was characterised by aging of $nTiO_2$ along with Cu and in a combination with NOM while the Type 2 aging condition included aging of $nTiO_2$ in a combination with NOM and Cu was freshly added to the aged dispersion prior to the exposure of the test

organism, i.e. *Daphnia magna*. The actual toxicity experiment followed the OECD 202 guideline. The results of the experiments applying the aging condition of Type 1 showed that irrespective of the aging duration (excluding 0 day aging), the presence of 2.57 mg/L $nTiO_2$ elevated Cu toxicity by ~ 2-fold as compared to an unaged Cu solution. In contrast to this, the presence of NOM in combination with 2.57 mg/L $nTiO_2$ showed a 2-fold reduction in Cu toxicity. The elevation or reduction in Cu toxicity in presence of 0.56 mg/L $nTiO_2$ in combination with NOM showed a similar trend but were often statistically not significant. In the case of the Type 2 aging condition, the presence of 2.57 mg/L $nTiO_2$ reduced Cu toxicity by ~ 2-fold while the reduction in Cu toxicity in presence of 0.56 mg/L $nTiO_2$ was by trend again comparable but not statistically significant. In addition, the presence of NOM in combination with 2.57 mg/L and 0.56 mg/L $nTiO_2$ diminished Cu toxicity up to 3-fold and ~ 2-fold, respectively. In consequence the present study shows that both presence of NOM and Type 2 aging condition assist $nTiO_2$ to alleviate Cu induced toxicity.

WE104

Estimating the distribution of TiO2, CeO2, ZnO and SiO2 nanoparticles with various forms when released to waste water treatment plant through sewers

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In recent years, there have been many challenges in developing environmental exposure model for estimating behavior of engineered nanomaterial (ENMs) as the production and use of ENMs increase. Most developed models are based on the assumption that ENMs release into the environment as their pristine forms and also remain in that state throughout their life cycle. However, it is reported that physical or chemical transformations of ENMs are inevitably occurred during their life cycle. Therefore, it is very important to predict the fate and transport of the ENMs in the intermediate pathway and it is also significant to estimate distribution of various forms of ENMs before releasing to environment. These results should be incorporated into exposure models as input data of emission amounts in order to improve reliability of predicted environmental exposure values. In this study, we compare the behaviors of the four kinds of engineered nanoparticles (ENPs): TiO_2, CeO_2, ZnO and SiO_2 when flowing in the sewers and the proportion of the forms of each ENPs before flowing into waste water treatment or natural water by using SWNano model. The SWNano (Sewer Water Nano) model was developed to predict behavior of ENPs in sewer system, which is representative intermediate pathway between emission and water compartment. And it was confirmed in our previous study that heteroaggregation process of TiO_2 ENPs with SPMs in sewage was the most influential process and rate of heteroaggregation was determined according to various conditions such as properties of sewage, ENP and sewer. In order to assess the behavior and the forms of not only TiO_2 ENPs but also other kinds of ENPs in the sewer, the physicochemical properties of these four ENPs such as size and zeta potential, etc. were obtained through experiment. Particularly, the size of ENPs were measured by using three equipment: SEM, DLS and SP-ICP since the size of ENPs is major factors in aggregation process. Attachment efficiency of ENPs were estimated by implementing empirical values of ENP properties to XDLVO theory. The model predicted that how difference in physicochemical properties of ENPs affected temporal concentration changes and proportion of released forms, although heteroaggregation is a major determinant of behavior in sewer for all ENPs. Furthermore, expansion of various types of ENPs in SWNano model represent that SWNano model can be a good candidate for a exposure assessment tool of ENPs.

WE105

Toxicity of novel nano-based sensors for early detection of corrosion on marine organisms

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Corrosion is an environmental and economic global problem. The regular monitoring of corrosion in maritime structures is critical due to safety reasons. However, current monitoring techniques are destructive, complex, expensive and do not provide real-time information regarding the structure's integrity. The MarTERA project "SMARTAQUA" (ERA-NET cofund scheme of H2020) is focused in the development of nanolayers that can be applied directly on maritime steel structures, to provide eco-friendly, low-cost and real-time detection of corrosion degradation. This will be achieved by incorporating smart nano-structured sensors containing active compounds that can detect the presence of specific triggers associated to the beginning of corrosion (e.g. pH changes or presence of chlorides or Fe^{3+}), for instance, by changing its colour. Layered double hydroxides (Mg-Al LDH) and hollow silica mesoporous nanocapsules (SiNC) were used as nanocarriers of potassium hexacyanoferrate ($Fe(CN)_6$) and phenolphthalein (PhPh), respectively. As far as we know, effects of Mg-Al LDH and PhPh in marine organisms are scarcely (or not yet) studied while hollow SiNC and $Fe(CN)_6$ are reported to exert deleterious effects in some marine organisms. Therefore, the present study aimed at assessing the acute and short-term chronic effects of both novel nanomaterials (LDH-[$Fe(CN)_6$]; SiNC-PhPh), together with the unloaded nanocarriers (Mg-Al LDH; SiNC) and the active ingredients ([$Fe(CN)_6$]; PhPh). Exposure tests were carried out using the green microalgae *Tetraselmis chuii* and the crustacean *Artemia salina* following the OECD 201 (2011) and ASTM E1440-91 (2012), respectively, with minor adaptations. Exposure concentrations range varied according to the species and tested compound. All materials were structurally and morphologically characterized. Engineered nanomaterials and both free sensing compounds exhibited no acute toxic effects in *A. salina* (NOEC=100 mg/L). Nevertheless, all tested compounds, apart from Mg-Al LDH, caused significant growth inhibition on *T. chuii*. NOEC values ranging from 3.25 to 5 mg/L were calculated for PhPh, SiNC-PhPh and SiNC, while NOEC of [$Fe(CN)_6$] was half than its nano-form (1.25 mg/L). Although the proposed nano-based solutions are marginally less toxic than the free sensors, it is recommended the implementation of safer-by-design strategies to promote a decrease in their current toxicity (e.g. substitution of surfactants).

WE106

Toxicity of the C60 to marine macroalgae Gracilaria caudata (Gracilariales, Rhodophyta)

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In recent years, the increasing application of nanomaterials (NMs) in various areas as industrial and household, contributes to the growth of the global economy. However, the fate and effects of these nano contaminants have attracted the attention of the scientific community, as these compounds are likely to cause adverse effects, even at low concentrations, they can cause potential risks to exposed organisms to the environment and to human health. The fullerene C_{60}, the object of interest in this study, was discovered by Kroto et al. (1985), as an allotropic form of carbon, being a relevant molecule with respect to the production, scientific interest and engagement in the research due to its physical and chemical characteristics and properties that are intrinsic, such as size, surface area and agglomeration/dispersion capacity. This nanoparticle has a potential environmental risk due to the propensity to affect the rate of absorption and toxicity of other environmental contaminants, which enhances the deleterious effect of these mixtures on exposed organisms. This study aims to investigate the effect of C_{60} under the marine macroalga *Gracilaria caudata J. Agardh*, red macroalgae of the Rhodophyta family, which grows naturally along the entire Brazilian coast, being distributed from the state of Maranhão to Santa Catarina. The *G. caudata* macroalgae is important for industries that extract from them a gum called Agar, which is widely used in the food industry, pharmaceuticals, cosmetics, agriculture, and fertilizers. However, the exposure of this macroalgae to contaminants has serious implications and may potentially represent an entry of contamination into the chain through its direct consumption by organisms at higher levels. In this work, to measure the growth rate at a laboratory scale, it was applied an accessible, and sensitive method that uses imaging described by Alves-Lima et al. (2018), with adaptations. The stock solution of the fullerene will be diluted in seawater to the desired concentration. A preliminary test will be done in a geometric series of 5 concentrations, to determine the concentration range, delimited by the lowest and highest concentration of inhibition. This interval, in turn, will be used in the preparation of the definitive test. The Inhibition Concentration (IC50) of the algae growth will be determined.

WE107

Reactivity of ZnO nanoparticles in synthetic saliva: dissolution and transformation driven by phosphate

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The use of ZnO nanoparticles (nZnO) in personal care products, dentistry, food packaging, etc. raises a potential concern about the ensuing oral exposure. As point of entrance to the gastrointestinal tract, the oral cavity represents a compartment of major interest for studies of risk assessment of nanomaterials. Once in contact with saliva, nZnO may undergo –among others- chemical and physical transformations which include dissolution (to produce the free ion) and reaction (for instance, with phosphate anions). The fate and speciation of the introduced Zn will depend on all these transformations. Conventional techniques such as ultrafiltration (UF) combined with inductively coupled plasma mass spectrometry (ICP-MS) exhibit some limitations which can be overcome by the electroanalytical technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) [1]. This technique has successfully shed light on the kinetics and thermodynamics of nZnO dissolution in various media [2-3] as well as on the relevance of nZnO dissolution for toxicity tests *in vitro* [4]and *in vivo* [5]. One advantage of AGNES is that there is no need of a separation step. This presentation will focus on the study of nZnO evolution in contact with synthetic human saliva with several techniques, including AGNES. The key role of phosphate will be highlighted in the kinetic and thermodynamic results. For instance, in the presence of phosphates, nZnO dissolve at a reduced speed in comparison with the dissolution rate of nZnO in non-complexing solutions of the same ionic strength. Solubility of nZnO in saliva determined by AGNES titrations will be presented. AGNES methodology compares favourably with data obtained by ultrafiltration and inductively coupled plasma mass spectrometry (UF-ICP-MS) in terms of speed, reproducibility and access to the free metal ion concentration. References: [1] Galceran J, Companys E, Puy J, Cecília J, Garcés JL. 2004. J Electroanal Chem 566:95-109. [2] David C *et al.* 2012. J Phys Chem C 116:11758-11767. [3] Galceran J *et al.* J Electroanal Chem 722: 110-118. [4] Mu Q *et al.* 2014. Chem Res Toxicol 27: 558-567. [5] Adam N *et al.* R. 2014. Nanotoxicology 8: 709-717. Financial support from the Spanish Ministry MINECO (Project CTM2016-78798) is gratefully acknowledged.

WE108
Transformation/dissolution of cobalt nanoparticles in biological media - influence of adsorbed biocoronas of amino acids, polypeptides and proteins
N. Mei, KTH royal Institute of Technology
This study focuses on the interactions between cobalt (Co) nanoparticles (NPs) and biomolecules in phosphate buffered saline (PBS) to understand how the adsorption of biomolecules influences the dissolution characteristics. Amino acids with different functional groups (*i.e.* lysine, glutamine, glutamic acid and cysteine), oppositively charged polypeptides (poly-lysine and poly-glutamic acid) and proteins (lysozyme and mucin – relevant for the inhalation route) were selected to investigate how the different properties of the biomolecules influence the surface reactivity of Co NPs. This was accomplished by employing different analytical techniques and electrochemical tools. From this study it can be concluded that the smaller biomolecules (the amino acids) do not adsorb on the Co NPs in PBS, whereas adsorption takes place for the larger biomolecules (polypeptides and proteins). This can be explained by the high affinity between Co NPs and phosphate ions in PBS, which results in the formation of Co-phosphate compounds on the surface that hinders the adsorption of the small amino acids. For the larger biomolecules, the driving force of adsorption is higher due to an entropic gain (*i.e.* increase of the free energy) originating from the exchange of small molecules (*e.g.* water and counter ions) from the Co NP-biomolecule interface. The formation of Co-phosphate at the Co NP surface reduces the protective properties of the surface oxide of the Co NPs, as evident from a reduced open circuit potential (OCP) value (reduced nobility). Except for cysteine, adsorption of the biomolecules had minor influence on the dissolution of Co NPs compared to dissolution in PBS only, though some kinetic differences were observed. Enhanced dissolution in the presence of cysteine is believed to be associated to the relatively high affinity between cysteine and Co-ions in solution, reactions that chemically drive the dissolution process. The adsorption of the larger biomolecules initially (< 4 h) resulted in reduced Co dissolution, but did not influence the long term behavior. The dissolution of the Co NPs in biomolecule-containing PBS solutions was very fast, with 35-55% of the Co NPs dissolved within one hour in the amino acid-containing solutions, and 15-40% in the polypeptide and protein solutions, similar to findings in PBS only. The results indicate that Co NPs will readily dissolve and only exist as NPs for limited time periods at biological conditions, *e.g.* in the human lung.

WE109
The standard development of nanoparticle exposure assessment through SP/ICPMS equipment
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The development of nanotechnology, particularly of nanoparticles, is having a revolutionary effect in science and technology. It is important to develop with suitable physical and chemical properties of nanoparticles. Otherwise, it receives unexpected adverse effects (e.g. ecotoxicological) which can be caused by the special properties of the nanoparticles. So there is requiring a study on the safety and the risk of the nanoparticles as well as the development of nanotechnology. In recent years, toxicity assessment method using nanoparticle in stable suspension has been emphasized in safety evaluation of nanomaterials. The nanoparticles dispersed in the aqueous solution have the possibility of creating an artifact that does not reflect the state of the dispersion aqueous solution. Confirmation of the conditions for measuring the size and size distribution reflecting the state of the dispersion aqueous solution is fundamental to reliable nanomaterial characterization. In addition, this characterization is needed to design environmentally appropriate toxicity studies and risk assessments. In this work, the size and size distributions of liquid suspension containing well-dispersed standard nanoparticles are measured by single particle inductively coupled plasma-mass spectrometry (SP/ICP-MS). The advent of SP/ICP-MS has helped advance the field of nanometrology, specifically at concentrations and in matrices that are environmentally relevant. SP/ICP-MS is a technology that can be applied to metal nanoparticle analysis of 20 nm or more. The required concentration in the sample has the advantage that it can be analyzed sufficiently below the ppt level. The size distribution measurements of particles are made using variety certified reference materials (CRM). These were compared with certificate values. And we have found reliable measurement conditions through repeated measurement. Through these results, the new environmental fate and exposure model of nanoparticle in different matrices can be developed through these results which contains relationships between agglomeration rates, primary size, and surface charge of the nanoparticle and the matrix.

WE110
SETAC Nanotechnology Interest Group
C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology
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Improving Decision Support for Sustainability Going beyond Standard LCA (P)

WE111
Using customised agricultural LCA tools in research projects: reconciling ease of use and flexibility. The case of cropping system modelling
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Customised agricultural LCA tools are increasingly popular, because they simplify LCA studies of agricultural production systems. These tools are easier to use than generalist LCA tools and reduce the risk of errors. However, compared to more generalist LCA tools, customised LCA tools are less flexible, and it is harder to tackle new research questions, that were not anticipated during the design of the tool. This study shows that customised LCA tools can be used to address new research questions, provided that they are adapted to them. The customised LCA tool MEANS-InOut was developed in a "product-oriented" way, to answer the question "What are the resources needed and the emissions due to the production of the agricultural product X?" This question resulted for crops in the scope definition: one product, on one field, for one year. LCA studies considering a single crop are challenged by agronomists, because interactions among crops within the rotation, which are a key factor for cropping system design, are largely ignored. The ACV Bio project has the ambition to address this question. In a cropping system view, the scope of the system is: several products, on one field, for several years. MEANS-InOut has been chosen as the tool to create LCIs in this project A cropping system is defined as the crops, crop sequence and management techniques used on a field over a period of years. It could be represented as the aggregation of the crops composing it. Considering this, no adaptation of LCI modelling is necessary. However, this approach does not take into account the interactions among the crops. The need for methodological and tool adaptations to assess the effects of the interactions among the crops of a cropping system was recognised in the planning of the ACV Bio project. Agronomists and tool developers worked together to adapt MEANS-InOut to the study of a cropping system as whole. These adaptations profoundly changed the data entry forms, emissions models and export functions. It also allowed automatic consistency controls and improved the tool usability. This case-study shows that a customised agricultural LCA tool can be used to answer a new research question, provided that the tool has been initially designed to be adaptable and that time and resources for the adaptation of the tool have been planned in the project. Strong interactions between scientists, users and tool developers are key to deliver tools, adapted to the need of users.

WE112
Integrated Life Cycle Analysis and Multi-level Energy Systems Modeling
E. Gencer, Massachusetts Institute of Technology; S. Torkamani, ExxonMobil Research and Engineering; F. O'Sullivan, Massachusetts Institute of Technology
The global energy system is undergoing a major transformation facing the dual challenge of meeting increasing energy demand while reducing greenhouse gas emissions. This change is characterized by the convergence of power, transportation, and industrial sectors, and the surge of multi-sectoral integration. Understanding the implications of these dynamics is challenging and requires a holistic approach to provide systems-level insights. To address this need, we have developed a systems-level life cycle analysis (LCA) framework that is designed to explore the emissions impacts of relevant technological, operational, temporal and geospatial characteristics of the evolving energy system. The underlying analytical engine includes the cradle-to-grave life stages of major energy conversion routes and covers more than 900 individual pathways. Detailed process simulation capabilities have been incorporated for in-depth analysis of greenhouse gas emission sources such as power plants and selected chemical conversion pathways. In addition to performing conventional LCA, we have implemented models for vehicle fleet and electric power systems to analyze systems-level interactions. By executing the analysis with embedded fleet models, we can establish a basis for the accurate assessment of the life cycle implications arising from complex system-level restructuring. The presentation will focus on the modeling methodology and the results of case studies.

WE113
Life cycle assessment, eco-design and recyclability of infrastructure integrated flexible thin film solar cells
J. Mahaux, HELMo-CRIG
Public infrastructure represents a huge area of built environment that can be used

for renewable energy generation by integrating solar cell materials into road pavements and road furniture. As electric driving is rapidly developing, and the use of intelligent traffic control systems is growing, an increasing amount of this infrastructure produced energy can be used locally. Moreover, it can enhance secure operation of critical control systems independent of external power failures, and on remote locations local energy generation can be more cost effective. Promising demonstrators have already been realized where crystalline solar cells are incorporated into bicycle paths, roads, smaller demo projects and sound barriers. However, costs of such systems are still high, not only because they have not yet developed towards the stage of standard high-volume products, but also because they are based on brittle silicon solar cells in the form of small tiles. These require severe mechanical protection and much assembly. This project aims to reduce cost by using flexible (and thus less breakable) thin film solar sheets, and by more effective integration of long lengths of these into public infrastructure. As a result, large scale durable electricity generation without additional land use will be enabled close to point of use. In order to ensure and support the environmental sustainability of renewable energy generation with infrastructure integrated thin film solar cells, a comparative life cycle assessment is performed. The considered incumbent technology is crystalline silicon solar cells while several emerging flexible thin film technologies are investigated: CIGS, CdTe and perovskite solar cells. Direct partnership with research centers and companies developing the emerging technologies enables the collection of as many site or laboratory specific data as possible. On the other hand, the main data sources for silicon solar cells are existing databases. Prospective efficiency models, that will be adapted throughout the project, are used to investigate multiple scenarios. As industrial scale development is not yet reached, the impact assessment results can therefore be used for recommendations on eco-design of the infrastructure integrated thin film solar cells, the recycling process to adopt and future development needs. This project is supported by the Interreg V-A Euregio Meuse-Rhine program.

WE114

Life Cycle Environmental Impacts of Synthetic Diamond Production

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Synthetic diamond is a superhard material found in applications such as cutting and grinding of rocks, minerals, metals and plastics, but also in windows and lenses in analytical equipment. Since the 1950s, when the synthesis of diamond via the high-pressure high-temperature (HPHT) synthesis was introduced, the use of synthetic diamond has gained increasing importance within industry, for example in cutting processes. Today, synthetic diamond dominates the industrial diamond use over its natural counterpart and the production is mainly via HPHT synthesis. Chemical vapor deposition (CVD) synthesis is also applied but to a lesser extent due to various technological issues, including the tendency of CVD diamond coatings to wear and tear in tooling applications. Nevertheless, a lot of research exist in the area of synthetic diamond film production via CVD synthesis addressing technological issues and CVD diamondhas been described as the potential facilitator of a new diamond age. In the strive for sustainable production, it is important to investigate the environmental impacts associated with various products and life cycle assessment (LCA) is commonly applied for this purpose. Until now, however, no study has conducted LCA of conventional synthetic diamond production via HPHT synthesis and so far, only laboratory-scale LCA results have been presented for one CVD synthesis alternative. The aim of this study is to conduct a cradle-to-gate LCA for synthetic diamond production both via the conventional HPHT synthesis and the potentially emerging CVD synthesis. For the latter route of synthesis, prospective (or ex ante) LCA was conducted and a predictive scenario was constructed focusing on microwave CVD. Future scenarios were constructed, e.g. by modelling the background energy system both using a fossil-based mix, representing the currently dominating Chinese production, and a renewable energy mix in an explorative scenario. A comprehensive uncertainty analysis was furthermore conducted for the included parameters. Environmental impacts were compared between the two routes of synthesis and hotspots in synthetic diamond production identified. The results from this research can be applied as a foundation for further LCA studies of synthetic diamond products.

WE115

Use of LCA methods for organisational decision-making. Case study of the Organisation Environmental Footprint (OEF) and the Nordic Swan Ecolabel in the retail sector

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The Organisation Environmental Footprint (OEF) methodology aims to outline specific, prescriptive methodological standards to be used in the European Union in order to assess the environmental impacts of organisations. It should be a multi-criteria, life-cycle-based approach that considers all organisational and related activities across the supply chain, provides for reproducibility and comparability over flexibility, and ensures physically realistic modelling. In this study we compared the OEF methodology to the approach of the Nordic Swan Ecolabel. The Swan, established in 1989, is well-known within the Nordic market and grants a license for the best performing products or services in a particular category, using existing LCAs and life cycle thinking based methods as sources for the environmental aspects focused. There are differences between OEF and Swan in the way the functional units and reference flows are defined. The Swan uses shares of sales, number of products available and sales in local currency. OEF measures the quantitative amounts produced and eventually consumed based on sales and losses occurring at the retailer, together with the environmental impacts of each product. OEF analysis is an aggregation of all the products of an organisation, which for the purposes of the Organisation Environmental Footprint Sector Rules (OEFSR) is a virtual, average retailer in the EU market. OEF also measures the energy consumption of the store and its impacts. The OEF shall include all upstream impacts, and some downstream ones if possible. The Swan includes only aspects related to production and use of goods sold in the shop and waste production and sets its system boundaries based on relevance, potential and steerability of the label. From a retailer's point of view, both the schemes act more as environmental management systems than as a way of external communication and gaining added value in the market. As opposed to the Swan, OEF does not set any requirements for performance levels. Therefore, it could be easier for the store to adopt, but on the other hand, it does not indicate good performance externally like the Swan does. Thus, OEF would be a helpful tool to gain a broad information basis, based on multiple environmental criteria and including upstream activities. It is time to consider using OEF in the criteria development of type 1 ecolabels like the Swan, and as an information basis for the environmental management systems of organisations.

WE116

Evaluating climate change pathways through a building's lifecycle based on dynamic impact assessment metrics

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A current practice of Life Cycle Impact Assessment (LCIA) uses a finite analytical horizon time and steady-state modelling conditions to provide traditional characterization factors. Metrics for dynamic environmental assessment and its computational tool have recently been proposed as an alternative solution to overcome these limitations dues to lacking of time dimension in LCA, precisely for climate change and toxicity impact categories (A. Shimako et al., 2017). As a building system has a very long lifetime, a framework of dynamic LCA method specific to the building sector was recently proposed by K. Negishi et al. (2018), allowing supporting the sustainable building design by calculating environmental burdens and the associated impacts on climate as temporal functions. The main purpose of this study is to identify building-related industrial sectors as hot spots with the aim to introduce an effective policy instrument of GHG mitigation actions for building's stakeholders. The dynamic LCA methodology is applied to a testbed case on a French building. This advanced dynamic approach is performed by integrating parameters that differentiate temporally LCI and LCIA and by combining traditional and new LCA tools (e.g. matrix-based LCI modeling method, database, allocation method, etc.). In this work were evaluated by dynamic LCA environmental impacts of 10 categories of construction products and compared with conventional LCA results in order to see how dynamic LCA would complete conventional LCA and support decision makings. The graphical representation of dynamic LCA results as a function of time provides important features proposing the proper consideration of essential information for time periods encompassing the lifetime of the studied system. Following anthropogenic activities over time, the dynamic profile of impacts allows making an appropriate decision at each occurrence of environmental burdens. Through the case study, it is shown that the complete dynamic LCA framework can be successfully applied to a building system considering system evolutions over time. The main findings from this work are that temporally distributed emissions give a completely different perspective of impacts and the dynamic metrics show the importance of setting targets for mitigation efforts concerning long-term emissions such as construction products requiring recurrent replacement to attain our objective of limitation of the global warming.

WE117

Sustainability performance of emerging upcycling applications of silanized fly ash reinforced polymer composites from WtE (Waste to Energy) plants

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WtE (incineration of municipal solid waste for energy recovery) is a sustainably preferred waste management strategy compared to landfilling. This energy is utilized to meet municipal and/or industrial electricity and heat demands of the society. Bottom and fly ashes are two important byproduct streams of WtE incineration plants. Bottom ashes are non hazardous in nature because of their lower heavy metal concentration and being increasingly used as secondary construction material .On the other hand, fly ash from WtE plants is hazardous and European Union mandates its disposal in hazardous waste landfills. Potential avenues for recycling fly ash can be classified into three types: (a) separation methods (b) solidification/stabilization and (c) thermal methods, which are used in construction industry (asphalt filler, cement clinker etc.). Although recycling fly ash is encouraging, its value as a secondary (i.e. recycled) material in the economy is not significant. Recycling of secondary materials derived from waste streams into avenues with high economic importance is strongly recommended according to principles of circular economy. This study evaluates sustainability performance of three emerging upcycling applications of silanized fly ash reinforced polymer composite (immobilization of heavy metals in ash and up scaled as filler reinforcement in thermoplastic and thermoset polymers) as: (a) thermal insulating false ceiling roof that potentially reduces air conditioning load of a building; (b) lightweighted automotive parts that improves fuel economy of an automobile; and (c) as a coating for protection of mild steel from atmospheric corrosion. The sustainability performance of these applications is determined in two steps. Firstly, their environmental performance is evaluated using a life cycle assessment (LCA) study. Secondly, a screening level life cycle costing analysis is performed by including environmental externality costs (or savings) that are quantified based on LCA results. The assessment is aimed to improve understanding on sustainable benefits and implications of silanized MSW fly ash reinforced polymers considering their properties of thermal insulation, mechanical strength and corrosion resistance. The outcome of this work offers critical insights to multiple stakeholders, such as local municipalities operating WtE incineration plants, venture capitalists with stakes in promoting sustainable innovations and technologists of the field.

WE118

Life Cycle Assessment of Added Value Compounds Production with Microalgae

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Microalgae are an alternative to produce sustainable fuel, food and high-value compounds such as carotenoids and terpenes for nutraceutical supplements and cosmetics. Algae production is focusing on these high-value compounds, as their selling price can guarantee the return on investment and they are bio-based ingredients. Against this background, the EU-project ABACUS was developed. Within this research project, we are assessing the environmental impacts of the technologies applied to produce carotenoids and terpenes by Life Cycle Analysis (LCA) methodology. We investigated the impact of algae cultivation on the LCA results by comparing the three most relevant photobioreactors (PBR) available on the market: the flat panel airlift (FPA), the horizontal tubular unilayer PBR (HTU-PBR) and the green wall panel (GWP). We also started to analyze the downstream processes (harvesting and extraction) and then we studied the influence of the characteristics of the PBR location by carrying out a sensitivity analysis for both indoor and outdoor cultivation in different European regions. Our preliminary LCA results based on data from pilot scale production indicate that location has a significant impact due to different algae productivities and energy demand for lighting, heating and cooling. We found that in particular for indoor systems, the electricity consumption is the main contributor to the GWP and that the related impact would decrease significantly if the share of renewable energy would increase. Moreover, our results indicate, that a crucial issue for assessing the algae technology is that processes at laboratory and pilot scale are not optimized and unfavorable LCA results are due to a poorly designed system. For this reason, we tried to develop together with engineering experts an upscaling methodology applicable for the different cultivation systems. Already with our first LCA results, we could successfully start to exchange ideas and advise our industry partner. We found that the results of our sensitivity analysis helped technology developers to understand to which extent the different stages of the process influence the results and how the environmental impacts could be reduced by changing the system configuration or energy sources. The results are based on the project ABACUS, which receives funding from the Bio-Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program (Grant Agreement N° 745668).

WE119

Use of life cycle assessment in process development of a catalytic fast pyrolysis method for bio-oil production

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To meet future demands of energy from non-fossil sources, biofuels has been pointed out as important. To generate liquid fuels from biomass, fast pyrolysis is one of the most promising technologies. However, the produced pyrolysis oil may have several major drawbacks which suppress its application for power and heat generation or transportation fuels. These are: high oxygen, water and water-soluble (acids) contents which affect negatively the acidity (corrosion effect), miscibility with petroleum-based fuels (separation of fractions), chemical stability (aging), high viscosity and low energy density. In the EnCat project, a new process is developed that will make it possible to use both woody biomass and biomass residues from agriculture for production of bio-oil via pyrolysis. The drawbacks mentioned above will be avoided through a novel biomass pre-treatment step followed by pyrolysis in a reactor making use of deoxygenation catalysts. Simultaneously, CO_2 will be captured with sorbents and hydrogen will be produced. After cleaning, the oil vapours will be mildly hydrogenated to produce a high-quality bio-oil. Life cycle assessment was implemented early in the process development process as a method to identify environmental hotspots. Also, which applications for combustion of bio-oil that might be the most suitable in order to optimize overall environmental effects were identified. It was found that one hotspot was the pre-treatment step and from the LCA results, it was suggested that further process development of the pre-treatment step was focused on chemical and water recovery. Another hotspot of the process was catalyst use. Therefore, an advice for the researchers developing the process was to work on regeneration of the catalyst.

WE120

Integrating future background scenarios for prospective LCA - method and case study on the German energy system

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Highly detailed bottom-up energy system models are often used for evaluating future energy system configurations from an infrastructural and systematic perspective. However, the integration of environmental impacts next to directly emitted CO2 into those models is largely missing. Albeit, it would offer the opportunity to identify highly detailed system configurations that are both cost-efficient and ecologically sustainable from a broader perspective. In order to ensure the holistic assessment of the ecological sustainability of systems, life cycle assessment (LCA) appears to be the most important and appropriate instrument/methodology. While LCA is based traditionally on static life cycle inventory (LCI) models (e.g. based on today's energy mix), we develop a general framework for deriving prospective LCI-based impact coefficients for energy technologies (electricity, heat, synfuels, mobility) from a detailed bottom-up LCI. The framework a) breaks down LCI coefficients into the life cycle phases of construction, operation and decommissioning and thus enables the allocation of environmental impacts construction, operation, and end-of life to the individual model years and b) integrates global background scenarios for power generation into the life cycle assessment of energy system relevant technologies, resulting in strong changes of specific environmental impacts in some impact categories. c) The framework furthermore allows analysing the demand for critical minerals for future energy technologies (not on a life cycle basis). The modelling framework is applied to transformation paths of the German energy system up to 2050. Expected results show that the climate friendly (with respect to direct emissions) transformation of the energy system will result in decreasing environmental impacts in some impact categories (such as human health), but increase environmental impacts in others (such as resource demand, in particular with respect to land use and critical minerals).

WE121

Scaling-up techniques used in prospective life cycle assessment of chemical technologies - a review

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Life Cycle Assessment (LCA) has been successfully used as a framework for the environmental assessment of mature chemical technologies. However, applying LCA to novel technologies is a challenging task. One challenge is that LCA is performed to assess emerging technologies being developed at the laboratory/pilot scale and these technologies are not yet commercially available. The industrial scale processes differ from those at the laboratory/pilot scale. By having complex equipment and sophisticated features, e.g. heat recovery systems and waste recycling loops, the industrial processes are more efficient than the laboratory/pilot scale processes. The issue is that for the emerging technologies no industrial scale data exists, which is necessary for conventional LCA. In some cases, the lack of industrial scale data is resolved by using scaling-up techniques. Here, we present a review of the scaling-up techniques applied in prospective LCA of the chemical technologies. The aim of the study is to review the scaling-up methods and classify them into groups. Different name variations for LCA of emerging technologies were used as the keywords in the literature search using Web of Science and Scopus academic search engines, e.g. "prospective LCA", "ex-ante LCA", and "anticipatory LCA". The number of the LCA publications relevant to this study was 53. The titles and the abstracts of those works were screened, and 15 prospective LCA studies were selected. Different scaling-up methods were applied in these LCA works, e.g. the use of scenarios based on thermodynamic calculations, proxies from the technologies at the industrial scale and the use of the optimization software, e.g. Aspen. The review work is ongoing, and in the next step the common features of those scaling-up techniques will be identified and then the methods will be classified into groups. The results of this review work could be used by the LCA practitioners aimed to scale-up new chemical technologies in prospective LCA. **Keywords:** review, prospective LCA,

emerging technology, scaling-up, optimisation

WE122

Life Cycle Assessment in support of the benchmarking of metals production techniques: approaches for dealing with multifunctionality and implications for decision-making

F. Lai, F. Laurent, J. Villeneuve, Y. Menard, BRGM / Waste and raw material unit

The demand for metals is expected to follow an exponential increase in the years to come to satisfy an ever-growing market for manufactured goods. In the meantime, the depletion of metals-rich deposits requires to develop new beneficiation and extraction processes in order to recover metals from low-grade/complex ores (e.g., hydrometallurgical processes such as bioleaching). In addition to technical and economic performances, the environmental sustainability of such new techniques must be assessed. For this purpose, Life cycle assessment (LCA) is a widely accepted tool that also allows comparison of different options. Nevertheless, such comparison is not always straightforward, since the recovery of metals from polymetallic ores results in the production of several metals of interest, thus raising multifunctionality issues. The ISO standards describe different allocation criteria/substitution approaches to deal with multifunctionality in LCA, which can lead to significant differences in impact assessment (LCIA) results. The goal of this study is to compare and discuss the different approaches for solving multifunctionality in the context of techniques benchmarking through a LCA case study. Two hydrometallurgical routes have been considered: atmospheric leaching and pressure leaching. The processed materials are two samples from a same bulk concentrate that eventually yield three metallic co-products: copper, zinc and lead. As copper is assumed to be the main metal in this case, the functional unit has been set as “the production of 1 ton of copper”. The system boundaries include the leaching step and the downstream recovery of the three metals. To apportion the environmental burden to the copper (with respect to the functional unit), five multifunctionality-solving scenarios have been implemented in accordance with the ISO 14044: (i) partial subdivision combined with mass allocation; (ii) substitution by system expansion; (iii) mass allocation; (iv) economic allocation and (v) allocation to the main metal (i.e. copper). The discrepancy in LCIA results with respect to the five scenarios confirms that the choice of the allocation/substitution rules implemented affect the LCA conclusions. This should be studied through sensitivity analysis during the life cycle interpretation as it can strongly influence the subsequent decision that is to be supported by the LCA.

WE123

Sustainable concrete through CO2 mineralisation: an environmental analysis of the different available options

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Portland cement (PC) is the most used binder in concrete production, and it is responsible for 8-10% of the global anthropogenic CO_2 emissions. PC is also accountable for 74-81% of the carbon footprint of concrete. Therefore, substantial climate impact reductions for concrete will require eco-innovation at the cement level. Today, one of the most promising technology to reduce PC production is its substitution with industrial by-products. However, by-products availability is rather constrained. Therefore, future sustainable cements must rely on a wider variety of solutions, and the construction sector will have to be more diverse and tailored to local resources, depending on the availability of secondary raw materials from different industrial plants. In this context, mineral carbonation represents a promising option to both mitigate atmospheric greenhouse gas concentrations and to produce sustainable concrete. A suitable class of materials for carbonation are alkaline slags generated as by-products from stainless steel production industry, so-called stainless-steel slags (SSS). The study focuses on the carbon footprint of new concrete made through mineral carbonation of SSS. Three examples of SSS carbonation process are analysed, each using a different source of CO_2: flue gas from the steel industry, in which CO_2 is separated through a cryogenic process biogas from anaerobic digestion, in which CO_2 is separated through membranes waste gas from ammonia production, in which CO_2 is separated through chemical absorption The produced carbonated concrete is equivalent in performance to traditional PC-based, non-reinforced concrete products on the market. By using the life cycle assessment methodology, the study describes the environmental performances of carbonated concretes made using different sources of CO_2 and highlights the environmental hotspots of carbonated concrete compared to traditional PC-based concrete. The carbonated concrete presents lower total CO_2-equivalent emissions compared to the traditional PC-based concrete. Membrane separation of biogas is the technology presenting the lowest CO_2-equivalent emissions. Regardless of the CO_2 source, all considered carbonated concretes serve as CO_2 storage, since the CO_2-equivalent emitted during their production is lower than the CO_2 uptake. Therefore, the environmental results show the potential for the carbonation process to reduce the climate change impact of traditional concrete production.

WE124

Manufacturers' data vs literature data - a comparison of LCI and LCA results for wood-burning residential heating systems

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Life Cycle Assessments (LCA) can help companies to improve their products to become more sustainable. The quality of LCA results is critically dependent on the quality of inventory data, but in LCA practice, secondary data sources are often used where primary data would be appropriate. Using the example of biomass heating systems, this study contrasts LCA results from primary data that were directly collected from manufacturers with those obtained from the ecoinvent database as a source of secondary data. Three approaches are compared: 1. Secondary data only: The exclusive use of unchanged ecoinvent data for both combustion and infrastructure manufacturing impacts. 2. Hybrid primary/secondary data: Some combustion emissions and manufacturing inventory from manufacturers’ data; other combustion emissions retained from suitable ecoinvent data sets. 3. Primary data only: no supplemental ecoinvent data are used, only the manufacturers’ infrastructure and emission data. The assessment was done for a log wood stove (8kW_{th} nominal power) and a wood pellet boiler (20kW_{th} nominal power). The idea was to collect as much data from manufacturers as possible on stove and boiler production and for the combustion emissions. Two companies provided bills of materials used in stove and boiler production and some emission measurements during the operation (practice-oriented, realistic test conditions obtained from literature). For secondary data and upstream processes, the ecoinvent database versions 3.4 and 3.5, as integrated in the software GaBi version 8.7, were used. Manufacturers’ primary data was checked for completeness and plausibility. The bill of materials for production of the stove and boiler is detailed and extensive - in both cases, at least 95% of the total weight can be determined. Emissions of CO, NOx and particulates were determined during the operation. Small differences in the LCA results were found between the second and third approach, but both were substantially different from the first approach using exclusively ecoinvent-based data. However, for the log stove, a noticeable difference for GWP and HTP in the operating phase can be attributed to CH_4 and N_2O emissions, while only small differences occurred between the second and the third approach in several other categories (acidification, eutrophication, ozone depletion, and photooxidant formation potentials). In the poster, the differences in results will be further analyzed and discussed.

WE125

Life cycle assessment calculative practices in the Swedish biofuel sector: Governing biofuel sustainability by standards and numbers

M.A. Martin, IVL Sweden Environmental Research Institute / LCA and Environmental Management; D. Lazarevic, SYKE Finnish Environment Institute

Since the introduction of the European Union's Renewable Energy Directive (RED), biofuel-producing firms are required to perform life cycle assessment (LCA) based greenhouse gas accounting in order to fulfill part of directive's sustainability criteria. This study adopts the concepts of “governing by standards” and “governing by numbers” to understand the LCA practices of biofuel-producing firms and assess the critical moments of friction between these alternative modes of governance. We focus our analysis on the use of LCA in the Swedish biofuel industry, undertaking case studies on the use of LCA in four Swedish biofuel-producing firms and semistructured interviews with industry associations and governmental bodies. Results indicated that the RED not only influences what biofuel sustainability entails but also structures the calculative practices used to measure it. At the same time,\nour results point to friction between achieving regulatory compliance and improving biofuel sustainability.

WE126

Sustainability Evaluation for tourism and MICE in Japan focus on tourism type Using Hotspots Analysis

Y. Kitamura, Y. Ichisugi, H. Suzuki, N. Itsubo, Tokyo City University

This study is the first phase development of sustainability evaluation for tourism and MICE (Meeting/ Incentive Travel/ Convention/ Exhibition and Event) in Japan. This time we introduce the study framework and examine the analysis results of priority check using Hotspots Analysis (HSA) based on Life Cycle Assessment(LCA). Tourism and MICE industry is said to have significantly higher growth speed than other industries and expected to have a high economic ripple effect. A recent study pointed out that the global tourism industry has a great negative contribution to climate change. In addition, international targets such as Sustainable Development Goals (SDGs) encompass not only focusing on climate change, but also considering some other topics. Therefore, our opinion is multiple criteria evaluation is needed in this industry. It follows LCA method can evaluate and analyze multiple effects and use assessment based on the Input-Output Tables (I/O). By using the HSA method, it becomes possible to confirm the important sources of environmental Impacts before each event. Here is a summary of our results. Calculations of the environmental impact and CO2 emissions were made to show characteristic of Tourism and MICE in Japan.

Results showed domestic tourism is found to have a large impact on land use, climate change and air pollution. Life Cycle Assessment (LCA) from CO2 emissions shows domestic tourism is the highest contributor. In addition, impact of air transportation and accommodation services is found to be high. Percent impact from souvenirs such as cosmetics and confectionery is also high. Total impact of tourism and MICE showed calculated CO2 emission result is 7.54% of the whole of Japan (2015). This research shows in the future it is possible to make trial calculations easily for each update of the survey data on the amount of consumption provided by the JAPAN TOURISM AGENCY (announcement of the latest year edition). We were able to show the same trend as in a recent study. Our study found not only air transportation and accommodation services, but also cosmetics and confectioneries such as souvenirs were found to contribute to the environmental impact and CO2 emissions in tourism and MICE in Japan. It is necessary to consider ways to reduce environmental burden from transportation as well as accommodation services and souvenirs.Therefore, this study will become part of the foundation for the Japanese tourism and MICE industry to achieve world sustainability.

Environmental Monitoring and Risk Assessment of UV Filters in Aquatic Environments (P)

WE127

Responses of periphyton and benthic macroinvertebrate communities to 4-MBC in stream mesocosms

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Concerns about the effects of ultraviolet radiation (UV radiation) in human health have lead to the increased use of personal care products containing Ultraviolet-filters (UV-filters). These compounds are frequently detected in aquatic ecosystems worldwide at concentrations reaching up to 2.4 mg/Kg (dry weight) in freshwater sediments. Thus, it is expected that benthic macroinvertebrate communities and phytobenthos, especially those living in areas with high human pressure, are being negatively affected by exposure to these contaminants. The aim of the present study was to assess the ecological effects of 3-(4-methylbenzylidene) camphor (4-MBC), frequently detected in European aquatic environments. For that, indoor artificial streams were used, where a natural benthic invertebrate community was exposed to sediments contaminated with 4-MBC. Effects were evaluated regarding macroinvertebrate abundance, as well as leaf decomposition and primary production. Results show that the macroinvertebrates community and leaf decomposition rates were not affected by 4-MBC exposure, but strong reductions in primary production were observed. This study highlights the importance of higher tier ecotoxicity experiments to assess ecological effects of low concentrations of organic UV-filters within freshwaters.

WE128

Organic UV filters in the Mondego River, Portugal

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Organic UV filters are of emerging concern due to their potential adverse effects on ecosystems and the human health. They are used as ingredients in personal care products (PCPs) such as body creams, facial make-up and sunscreens to protect the human skin against UV radiation. Furthermore, UV filters are widely used in industrial products such as plastics, paints, and textiles to improve the photo protective properties. UV filters may enter the aquatic environment directly through recreational activities or indirectly through wastewater treatment plant (WWTP) discharges. Their presence has been shown in different environmental matrices (e.g., sediments, biota, freshwater, marine water), revealing their potential for persistence or pseudo-persistence. This study investigates the occurrence of organic UV filters in the Mondego River, which is the largest entirely Portuguese River (258 km length) located in central Portugal. The Mondego River is subject to discharges from several WWTPs. It is also used for recreational purposes due to several fluvial beaches, particularly during spring and summer time. In September 2018, water and sediment samples were collected at 20 stations along the final 60 km of the river, from Penacova until Figueira da Foz, where it enters the Atlantic Ocean. The samples were analyzed for 26 UV protecting compounds. The water samples were enriched and eluted using an automated solid-phase extraction (Freestyle Xana, LCTech, Germany) method. The freeze-dried sediment samples were extracted using an accelerated solvent extractor (ASE-350, DIONEX, Germany) method. The instrumental analysis was performed on an Agilent UHPLC-MS/MS system operating with dopant-assisted atmospheric pressure photoionization (DA-APPI). This study shows the occurrence of UV filters in the Mondego River for the first time. In the water phase, the widely used compound octocrylene (OC) and the hydrophilic substances Benzophenone-4 and 2-Phenylbenzimidazole-5-sulfonic acid (PBSA) were dominant and found in concentrations up to 270 ng/L, 220 ng/L, and 90 ng/L, respectively. In the sediment, mainly hydrophobic substances were identified at concentrations in the low ng/g dw range. UV filters enter the Mondego River through several input pathways. This reflects their disperse uses in PCPs and industrial products.

WE129

Benzotriazole UV Stabilizers in Urban Rivers - Behaviour During Storm Run-Off and Source Identification

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Benzotriazole ultraviolet stabilizers (BT-UVs) are a sub-group of benzotriazoles with 2-hydroxyphenol attachments and various alkyl substituents on the phenol ring. Because their structures impart broad spectrum UV resistance, they find use in the prevention of UV-induced discoloration and deterioration of a range of materials. Whereas BT-UVs have previously been detected in rivers and sediments, their dynamic behaviour during storm runoff events in urban streams is poorly understood. We measured BT-UVs in (i) suspended sediments during two rainfall events in the highly urbanized Mimico River watershed in Toronto, Canada, (ii) plastic debris collected from the watershed and (iii) in pre-production plastic pellets. The concentrations of BT-UVs in suspended solids from Mimico Creek were the highest ever reported in the peer-reviewed literature for this type of sample. In particular, the contamination is considerably higher than what has been reported for major rivers in Germany and highly polluted waters in Japan. Based on the widespread use of BT-UVs in sunscreens and as polymer additives imparting resistance to UV-induced ageing and degradation of plastics, we are exploring different potential sources of BT-UVs to Mimico Creek. The highly urbanized nature of the Mimico Creek watershed results in an unusually high amount of plastic waste within and close to the stream.Several items of plastic debris from the watershed showed high concentrations of UV238 (pylon, golf balls) and UV234 (artificial turf). Such items contain BT-UVs as additives and are likely sources of BT-UVs to Mimico. All other plastics collected from the watershed (plastic bags, food and beverage containers, etc.) and all of the pre-production plastic and fibrescontained negligible amounts of BT-UVs. Most previous investigations into the transport and fate of BT-UVs have focussed on industrial and domestic wastewaters as key sources of BT-UVs. We demonstrate that BT-UV contamination can be comparable, if not greater in streams that are not directly affected by such wastewater streams. When combined with the wide range of BT-UV levels measured in plastic products, it appears that informal disposal of selected products containing BT-UVs plays a prominent role in BT-UV cycling in highly urban rivers.

WE130

Are sunscreens the solution for UV radiation deleterious effect for human and the problem for the environment?

S. Diaz-Cruz, IDAEA-CSIC / Environmental Chemistry

Personal beauty and hygiene products market is an expanding and increasingly diverse market. Product categories include skin, dental, hair and nail care products, cosmetics, fragrances, bathing and shaving creams, among others. Sun protection is the most important segment of the sun care market with expenditure of 7.0 billion € in 2014, and EU countries accounts for 32 % of the world market [1]. Many ingredients of these products are considered as pseudo-persistent contaminants of emerging concern (CEC), as a consequence of their wide use and release into the environment and a deficient elimination in wastewater treatments. However, there is a lack of regulation regarding their occurrence in the environment. Major components of sun care products are organic and inorganic sunscreens (also known as UV filters). The organic substances have radiation absorption capability in the range 320-400 nm (UVA) and 280-320 nm (UVB), whereas inorganic sunscreens reflect and scatter the sunlight. In order to protect in this wide radiation region, more than one sunscreen are included in the products. Previous studies have reported not only the occurrence of sunscreens in the environmental compartments (water, sediments and biota), but also show that sunscreen agents are toxic for aquatic biota and are accumulated and biomagnified [2]. Many organic sunscreens, as well as TiO_2 at nanosized scale are endocrine disruptors to humans and wildlife, being toxic to corals, fish and other reef organisms. Beside this knowledge, the ecotoxicity effects that many sunscreens and their transformation products may display in short and long terms are mostly unknown. Moreover, despite these evidences a lack of inquiry into their toxicities, lack of occurrence data, and even in some cases lack of reliable analytical methods prevent accurately assess whether they pose a real and present danger to these habitats [3]. In this presentation main occurrence (water, sediments, fish, dolphins, seafood, bird eggs,…), toxicity and risk assessment studies within our research group will be shown to provide a wide and up to date picture of current knowledge and potential threat posed to the environment in relation to sunscreen agents.

WE131
Environmental Risk Assessment of UV filters in Freshwater and Marine Environments: State of Science and Challenges
I. Davies, Personal Care Products Council / Science
UV filters are ingredients in personal care products that protect consumers against the\nadverse effects of UV radiation. UV filters are also used as photoprotective agents in other products, such as\nplastic and apparel items. Organic UV filters have diverse chemistries and moieties. Inorganic UV filters are\nmetallic and can be nanoscale or non-nanoscale. UV filters such as benzophenones share chemistries with\nnaturally occurring compounds. For example, oxybenzone is a natural compound produced by higher plants. It\nis crucial to appreciate these various chemistries and sources when assessing environmental fate and effects of\nUV filters. \n\n Environmental exposure to UV filters occurs via point and diffuse pathways. Point exposure\noccurs when UV filters wash-off bathers. This includes exposure to coastal sediment when, for example,\naerosolized UV filters are applied to people on a beach. Diffuse exposure occurs when UV filters are washedoff\ndown the drain and enter the waste water system. UV filters have been detected at low ng/L to µg/L levels;\nmainly in the marine environment. However, more monitoring studies are needed before a clear picture of\nexposure can be painted; the first step in an environmental risk assessment. \n\n When UV filters enter the\naquatic or marine environment they are subject to various fate and transport mechanisms. Very little is known\nabout these mechanisms, yet such data are essential to understand the concentration and timing of potential\nexposure of organisms to UV filters and/or their metabolites/degradation products. Further fate and transport\nresearch is therefore required to establish partitioning of UV filters between water and organic phases, biotic\nand abiotic degradation pathways and bioaccumulation of these materials. In addition to the limited amount of\ndata regarding the concentration of UV filters in the environment, very few ecotoxicological data are available\nto assess whether UV filters are hazardous at environmentally relevant concentrations. Although some\nlaboratory ecotoxicological studies have been conducted for a handful of UV filters, many questions remain\nover the extrapolation of these studies to the environment. Overall, huge data gaps exist for UV filters with\nregard to exposure and toxicological hazards, including endocrine activity. More research is therefore required\nto fill these data gaps and establish the risk these materials pose to organisms residing in the marine and aquatic\nenvironment.

Recent Approaches in Establishing Linkages between Exposure Science and the Environmental Effects of Trace Organic Contaminants (P)

WE132
Environmental fate and behaviour of a Tribenuron methyl metabolite in different types of soil, and its influence on soil fauna
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Yearly 2.6 mln metric tons of pesticides contaminate the environment. Most of them, i.e. 85%, are used in agriculture and get into the soil. There is no doubt that pesticides play an essential role in maintaining high levels of food production, but on the other hand, they affect soil fauna and many biological processes involving it. Hence, it is extremely important to determine the dynamics of pesticide degradation in the soil and the impact of pesticides and their metabolites on soil fauna. For this study a tribenuron methyl metabolite whis was a representative of pesticides belonging to the sulfonylurea group, was selected. This group of pesticides commonly occurs in various herbicides commercially available in Poland. Sulfonylurea herbicides are largely employed for the control of many grasses and broad-leaved weed species in crop protection of vines, rice, citrus, corn, potatoes, and tomatoes. The aims of this study were to (i) determine the rate of degradation of the tribenuron methyl metabolite in three types of soils commonly found in Europe; (ii) assess the adsorption and desorption behaviour of the tribenuron methyl metabolite on five different soil types; (iii) determine the K_d, K_{oc}, K_{Fads}, and K_{des}; (iv) determine the influence of the selected pesticide metabolite on the reproduction of the predatory mite Hypoaspis (*Geolaelaps*) aculeifer, the earthworm *Eisenia andrei*, and the collembolan *Folsomia candida*. The obtained results, showned that the dynamics of degradation of the investigated metabolite of tribenuron methyl depended on the soil pH level, which was also confirmed in reproduction tests when artificial soils were used. The presented study provided diverse data on the toxicity to the predatory mite, Hypoaspis (*Geolaelaps*) aculeifer, the earthworms *Eisenia andrei*, and the collembolan *Folsomia candida.* Also it proved that these tests should be an element of the routine ecotoxicity testing.

WE133
Can shell alterations in limpets be used as alternative biomarkers of coastal contamination?
I.B. de Castro, Universidade Federal de São Paulo / Instituto do Mar; N.G. dos Santos, Universidade Federal de São Paulo
The assessment of levels and impacts related to inputs of hazardous chemicals in coastal areas is an important way for implement protective measures for aquatic environments. In this regard, mollusks are widely used to assess environmental contamination and ecotoxicity. Furthermore, recent studies proposed morphometric alterations in mollusk shells as good proxies for assessing adverse biological effects of chemical contamination in multi-impacted coastal zones. The present study evaluated the association among traditional biochemical biomarkers with biometric, morphometric, and elemental composition of *Lottia subrugosa* shells from three multi-impacted coastal areas in Brazil. The study was carried out in Todos os Santos Bay (BTS), Santos/São Vicente Estuarine System (SESS) and Paranaguá Estuarine Complex (CEP), using three sampling sites to seek contamination gradients in each area. Results showed that all biomarkers evaluated responded to environmental contamination, regardless the presence (SESS and CEP) or absence (BTS) of a gradient of contamination. In fact, the response found by the new proposed biomarkers (biometric and morphometric parameters) are consistent to the traditional biomarkers of exposure and effects (lipid peroxidation and DNA damage). Indeed, changes in elemental composition of *L. subrugosa* shells suggest that exposure to contaminated environments is probably responsible for the alterations detected. Due to the simplicity and lower cost of biometric and morphometric analyzes, these parameters seem to be a promising tool for future assessments, especially in areas where technological resources are scarce. The findings from the present study were observed in three aquatic systems distributed over a wide range of latitudes, which strongly indicates that gastropod shells reflect effects resulting from environmental contamination.

WE134
Toxicokinetic parameters causing the discrepancy between the measured and predicted bioconcentration factors of pharmaceuticals and personal care products in fish.
R. Tanoue, K. Nakayama, Ehime University; M. Kondo, National Fisheries University; K. Nomiyama, Ehime University; T. Kunisue, S. Tanabe, Ehime University / Center for Marine Environmental Studies
Bioconcentration of organic compounds is generally considered as the partition between water and the organism's lipid phases, and thus lipophilicity of chemicals can be the critical factor. Nevertheless, our previous field-monitoring studies on pharmaceuticals and personal care products (PPCPs) in fish showed that the bioaccumulation factors of some PPCPs largely differed from bioconcentration factors predicted on the basis of their lipophilicity ($BCF_{predicted}$). In the present study, we determined the uptake and depuration kinetics of 95 PPCPs in common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*), and Japanese medaka (*Oryzias latipes*), to examine the factors causing the discrepancy between the $BCF_{predicted}$ and the bioconcentration factors calculated from the measured PPCP concentrations in the water and fish ($BCF_{measured}$). Assuming a worst-case and multiple-exposure scenario, fish were exposed to treated municipal wastewater for 8 days followed by clean water for 14 days. Uptake (k_1) and depuration (k_2) rate constants were successfully determined for 22 compounds. When comparing $BCF_{measured}$ in the three fish species with the $BCF_{predicted}$, similar values were observed for more than 40% of these 22 compounds. On the other hand, $BCF_{measured}$ were 2−4 orders of magnitude lower than the $BCF_{predicted}$ for telmisartan, rebamipide, fexofenadine, and triclocarban in all the fish species. This was likely due to rapid metabolism and excretion of these PPCPs, as the calculated half-lives were < 5h. In contrast, approximately 20 times higher $BCF_{measured}$ than the $BCF_{predicted}$ were observed for haloperidol in medaka, which can be explained by a fast uptake rate (k_1, 510/day) and slow depuration rate (k_2, 0.19/day). Similarly, fast uptake rate (k_1, 44/day) and slow depuration rate (k_2, 0.21/day) of chlorpheniramine in tilapia plasma resulted in approximately 10 times higher $BCF_{measured}$ values than the $BCF_{predicted}$. The k_1 values of the two compounds were relatively high compared with other compounds regardless of fish species, implying a specific partition to non-lipid components. To develop BCF prediction models in fish, possible factors affecting the k_1 and k_2 of chemicals, e.g., partitioning to non-lipid components and hepatic clearance, should be quantified and applied. Our results highlight the importance of understanding toxicokinetics of PPCPs in fish for the full implementation of predictive toxicology approaches.

WE135
Biological activities of antidepressants in wastewater to fish monoamine

transporters
M. Ihara, M.O. Ihara, D. Kato, H. ZHANG, H. Tanaka, Kyoto University
Over recent years, growing numbers of human pharmaceuticals have been detected in effluents of wastewater treatment plants (WWTPs) and river water. Antidepressants are one of most commonly detected human pharmaceuticals, and concern about their potential risks to aquatic species has been raised. It is possible to measure the concentrations of selected antidepressants by chemical analysis, but such concentrations do not indicate the biological activity of the antidepressants in waters. To determine whether antidepressants in aquatic environments alter the behaviour of aquatic organisms, we must know the extent to which such organisms may be exposed to antidepressants as determined by the antidepressants' mode of action. So far, we have succeeded to detect biological activities of antidepressant in wastewater against human transporter (e.g., serotonin transporter (SERT)) by using the newl developped in vitro assay, antidepressant assay. However, until now biological activity of antidepressants in wastewater against fish has not been investigated. In this study, we aimed 1) to investigate whether human antidepressant can inhibit medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*) SERT by in vitro antidepressant assay, and 2) to measure the biological activity of antidepressants in WWTP effluents against fish SERT. We constructed plasmids which express medaka and zebrafish SERT, respectively. At first, we confirmed that antidepressants can inhibit not only human but also fish SERT. Interestingly, both medaka and zebrafish SERTs were strongly inhibited by antidepressant compared to human SERT. Next, we applied the in vitro antidepressant assay to WWTP effluent extracts. All wastewater extracts showed reduction of APP uptake for not only human but also fish SERT. Interestingly, medaka and zebrafish SERT were more strongly inhibited compared to human SERT. When activities detected in wastewater extracts were quantified as fluoxetine-EQ, which ranged from 500 to 1,000 ng-fluoxetine-EQ/L for human SERT, 1,000 to 2,000 for medaka SERT, and 800 to 1,100 for zebrafish SERT. These results indicate that at least molecular level, fish might be more seriously affected by antidepressants in water environment than human. When we discuss risks of antidepressants in environmental water to aquatic species, species differencies should be considered. In vitro antidepressant assay using fish SERT shown in this study is potentially useful tool to predict risk for fish. \n

WE136
Impact of water management on arsenic and cadmium accumulation by rice (Oryza sativa L.)
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The accumulation of As in rice is a public health concern since As is toxic to humans; in particular, inorganic As can cause many chronic diseases including cancer. Rice crops are prone to accumulating As, in part, due to the anaerobic soil conditions triggered by the traditional continuously flooded irrigation practice. It is also known that As accumulation in rice grains differs with the rice variety. In addition to arsenic, crops can also take up cadmium, which also presents high toxicity to humans. However, unlike As, the lowest concentrations of Cd are found in rice grown by flooding, but information, particularly on irrigation, is scarce on a field scale. The aim of the present study was therefore to investigate the impact of different irrigation practices on the accumulation of Cd and As in several rice cultivars. Eight rice *(Oryza sativa L.)* varieties were employed in field experiments with four water management regimes comprising flooded, intermittent flooding, localized or drip irrigation and saturation irrigation. Total As and Cd concentrations were determined in rice grain and soil. Additionally, in some samples, roots and straw were analyzed at maturity stages of crops growth. Our results confirm that total As concentrations in brown rice grains are higher than in white rice grains, with average values of 284 $\mu g.kg^{-1}$ and 167 $\mu g.kg^{-1}$ respectively. Our results also confirm that the adoption of less reducing conditions in the soil during the rice growth causes the reduction of the total As amount in rice grains. This effect appears to be small when changing to continuous flooding to saturation irrigation (average total As values of 312 $\mu g.kg^{-1}$ vs 224 $\mu g.kg^{-1}$) but becomes more significant when traditional irrigation was replaced by intermittent flooding or drip irrigation (mean total As concentrations were 163.6 $\mu g.kg^{-1}$ and 68.38 $\mu g.kg^{-1}$ respectively). Regarding Cd content in rice grains, average concentrations ranged from 6 $\mu g.kg^{-1}$ (continuous flooding) to 30 $\mu g.kg^{-1}$ (drip irrigation).

Adverse Effects of Chemicals on Host-associated and Free-living Microbiomes (P)

WE137
Diethylhexyl Phthalate Causes Alterations in Host and Microbial Nutrient Metabolisms: A Potential Obesogenic Mechanism
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The unprecedented rise in obesity prevalence over the last 50 years cannot be explained by poor diet and lack of exercise alone. Recent evidence suggests that ubiquitous environmental chemical contaminants, such as plasticizers, may contribute to the epidemic. Chemicals suspected to influence obesity are known as obesogens. Phthalate plasticizers have been implicated as obesogens, however, mechanisms by which phthalates contribute to obesity are unclear. Because the gastrointestinal (GI) system is the most frequent site of phthalate exposure, the effects of phthalates on the GI microbiome in conjunction with alterations in host molecular changes may contribute to the development of obesity. While evidence for phthalate exposure in the absence of other stressors is inconclusive, mounting evidence suggests co-exposure to phthalates and overfeeding may exacerbate obesity compared to overfeeding alone. Thus, we hypothesize dietary diethylhexyl phthalate (DEHP) exposure, in addition to overfeeding, results in microbial dysbiosis, which modulates obesity in combination with alterations in host metabolic processes. *Danio rerio* were assigned to a normal feeding regime, overfeeding, or overfeeding with DEHP exposure for 60 days to study the exacerbation of obesity by DEHP exposure. Following euthanasia, gut and fecal matter were excised for RNA and 16S microbial sequencing, respectively. Processes related to lipid metabolism and immune function were significantly altered in the overfed + DEHP group compared to both control and overfed. Additionally, the co-exposure of overfeeding and DEHP resulted in alterations in microbial β diversity, increases in Bacteriodes, and decreases in Fusobacteria and Tenericutes compared to control and overfeeding. Additionally, co-occurrence network analysis of 16S data revealed decreases in cluster size and fracturing of the network into unconnected components compared to DEHP-free treatment. Functional analysis of microbial sequences with PICRUSt revealed alterations in various processes related to lipid and carbohydrate metabolism, paralleling the changes observed in the host metabolism. These data reveal DEHP exposure intensifies metabolic changes in the host and microbiome compared to overfeeding alone, which may ultimately result in exacerbated obesity.

WE138
Do nano-biomaterials designed for drug delivery affects soil microflora?
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A biomaterial (BM) is defined as any material that has been designed to interact with biological systems for a medical purpose. Conventional BM are well regulated, however existing guidelines need to be extended to address specific nano related concerns. In the EU-project BIORIMA we investigated the ecotoxicity with a focus on soil microflora. NBMs (nanobiomaterials) excreted by treated patients can via sewage get into wastewater treatment plants and via sewage sludge applied as fertilizer to soil and soil microflora. Several materials designed for targeted drug delivery were tested on the potential ammonium oxidation activity (first step of nitrification) which proved to be a very sensitive test system for conventional nanomaterials. Additionally, the effect on basal and substrate induced respiration with two test systems was investigated. Due to the high number of various NMs a reduced testing effort is essential. The MicroResp™ test system for measuring microbial respiration, could be an alternative test system to conventional test devices. So far, it has not been used to study effects on soil microbial processes in a regulatory context, but EFSA (2017) recommends that its capacity in that respect is determined. We compared the results of a conventional approach (Sapromat; online measurement of microbial activity requiring soil amounts of at least 100 g soil dry matter per replicate) with the miniaturized approach (only two measurement times: start, end; about 300 mg soil per replicate). Conclusions: 1. Due to biological variability the investigation of several concentrations and several time points are necessary for an assessment of NBM on soil microflora. 2. For dispersed materials, the additional investigation of the pure dispersant is required. 3. An ecotoxicological effect of NBM cannot be excluded – however presumably only at high concentrations and thus does not have any environmental relevance; 4. To understand the changes in the soil microbial community, both microbial activities (respiration, nitrification) have to be considered. Depending on the activity, stimulation and inhibition can be detected for the same NBM in the different test systems. 5. There are obvious differences in the respiration results between the two test systems and these need to be studied in future research. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760928.

WE139
Effects of a biopesticide produced by Artemisia absinthium on metabolic diversity of soil bacterias
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Biopesticides are increasingly used to replace synthetic pesticides in pest control and those of plant origin have shown numerous advantages over conventional pesticides. However, very few studies analyze in detail the effects that these biopesticides can cause to the environment, especially on non-target organisms. This study focused on the aqueous extract (hydrolate) of *Artemisia absinthium. So*

far, hydrolates have been considered a mere by-product of the process of obtaining essential oils, but now their biological properties are generating attention because they can become a sustainable source of new biopesticides. Hydrolate of *Artemisia absinthium* showed recently nematicide properties. *A. absinthium hydrolate* effects were tested on a natural soil microbial community whose taxonomy was analyzed through 16S rRNA Gene Sequencing. The physiology of the community was only slightly modified, namely through an increased ability to degrade substrates, except amines and amides that have a mild decrease. These effects are more markedly only at 100% concentration. These results allow for a better understanding of the impacts of biopesticides in the soil environment as a pest management alternative. *The authors thank the financial support of MINECO-FEDER (CTQ2015-64049-C3-2-R), Gobierno de Aragón-FSE (GATHERS E39_17R). We thank J. Burillo and J. Navarro-Rocha for his generous cession of the extracts used in this study.*

WE140

Effects of ingestion of Cu- and Ag nanoparticles on endogenous microbiota and gut physiology in rainbow trout

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Nanotechnology is rapidly developing exploiting the unique properties the nanoparticles (NPs) display. Some NPs, such as Silver- and Copper-NPs display antimicrobial properties and this ability to kill or impede the growth of pathogenic bacteria is important in products like medicine and personal care products. Production and incorporation of these NPs into consumer products will ultimately lead to release into the environment and may lead to unintended exposure to organisms. A likely route of exposure is by ingestion where they may affect beneficial/protective bacteria in the digestive system, and lead to immunosuppression and potential negative health effects. We investigated the effects of manufactured Ag- and Cu-NPs (and their ionic bulk) (50, 500 and 2000 mg/kg food), in a dietary experiment, on the microbiome of rainbow trout (*Oncorhynchus mykiss*) and aimed at linking changes in the composition to the health of the fish with respect to direct damage to the intestinal epithelium and changes in gene expression in the gut tissue, with special emphasis on immunological response. Data from the exposures is still being analyzed. The information obtained from this study provide knowledge of host-microbiome interactions in fish; information that is useful for determining the effects of NPs on environmental health and subsequent risk assessment.

WE141

Effects of Microcystis aeruginosa cultures on the composition and functions of the gut microbiota in the medaka fish (Oryzias latipes)

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Over the past few years, host-microbial associations are intensively studied to better understand and grasp mutual involvements in their relationship. Many investigations highlighted diverse effects of environmental stressors on microbial communities of organisms, notably in ecotoxicology studies. Due to global changes and eutrophication, cyanobacterial blooms producing bioactive metabolites start to be increasingly frequent, abundant and persistent worldwide occuring mainly in freshwaters. So far, researchers focused their work, among others, on cyanotoxin effects on aquatic fish physiology. To our knowledge, no research has analysed bloom-forming cyanobacteria effects on fish microbiota yet. In this study, we investigate potential effects under a wide range of concentrations from a culture of clonal strain of *Microcystis aeruginosa*, the most observed cyanobacteria in freshwaters and producing a large set of bioactive components, on the composition of the fish gut microbiota. To this end, the freshwater model fish, the medaka *Oryzias latipes*, is exposed through microcosm and mesocosm approaches. A comparative analysis of 16S rRNA gene sequences and associated functions are performed to examine gut microbial communities and functionnalities in each individual at given concentration, besides histopathological examinations to assess hepatotoxic effects.

WE142

Impact of a contaminated environment on the microbiome of Mauremys leprosa, an endemic freshwater turtle of the Mediterranean basin

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Located at the interface between the host and its environment, the microbiota influences many processes in its host. Thus the assemblage host-microbiota, called holobiont, seems to be essential in the response of organisms to environmental changes. However, in a context of global change, variations in physicochemical factors are often privileged over ecotoxicological research. Particularly scarce are studies concerning host microbial communities in disturbed ecosystems. As part of our study, we focused on the impact of wastewater treatment plant (WWTP) effluents on the cloacal microbiota of the Mediterranean pond turtle, *Mauremys leprosa*. The core microbiome of this species, independently of turtle's location, was mainly composed of bacteria of the Deinococcaceae, a family known to be tolerant to multiple stressors. Moreover, a significant difference in the composition of the microbiota from individuals located at both sides of the WWTP was observed. The WWTP discharges had a negative impact on the bacteria diversity, notably by lowering the equitability. The composition of communities was also affected, both in their abundance and in the identity of the taxa observed. In particular, female turtles were more impacted by the WWTP than males, with a downstream microbiota highly populated by fecal bacteria of the Enterobacteriaceae. The results obtained concerning the microbiota of the Mediterranean pond turtle, the relative sensitivity of this species to pollution - which has been demonstrated from a ROS (Reactive Oxygen Species) study - as well as its ubiquitous presence in the Mediterranean basin, make of this species a new holobiont model to assess water quality in this region.

WE143

Investigation of antimicrobial activities of sodium and zinc pyrithione against microorganisms in water systems and assessment of their cytotoxicity by confocal and transmission electron microscopy

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Sodium omadine (sodium pyrithione, NaPT) and zinc omadine (zinc pyrithione, ZnPT) are biocides in the ecosystem with the risk of damaging mainly invertebrates such as algae, fungi, mollusks, macro fouler groups like plants and other living forms. NaPT have been using as inhibitors to control the growth and settlement of microbes, higher plant and animal species, especially on the underwater surfaces. ZnPT have been using for being an antifouling biocide as well as in the pharmaceutical applications such as antidandruff effect, seborrheic dermatitis**.** The aim of this study was to evaluate the antimicrobial efficacy of NaPT and ZnPT against various microorganismsin the environmental water systems by using disk diffusion methodto prevent its ecotoxicity. The cytotoxicity of the agents by MTT assay on NIH/3T3 (ATCC® CRL-1658™),the cell proliferation were evaluated and the viability percentages were calculated. For morphological changes, after the application of IC_{50}values of NaPT and ZnPT, all samples were stained and imaged under Confocal microscope and the changes in the cell's morphology were assessed. Changes in the ultrastructure of NIH/3T3 cells exposed to IC_{50}values of NaPT and ZnPT for 24 hours were evaluated by transmission electron microscopy (TEM). Statistical comparison of the samples was carried out by one-way analysis of variance for multiple comparisons using GraphPad Prism 7.0 for Windows. All biocides showed antimicrobial activity against all of the strains. The bactericidal effect of NaPT and ZnPT was reduced in direct proportion to the decrease in concentrations and all concentrations. NaPT was found more effective on *E. coli*than other microorganisms.All concentrations of ZnPT showed more antimicrobial effect on *Candida albicans* than other bacterial strains. 1,25% concentration of ZnPT showed the highest antimicrobial effect to all strains.In NIH/3T3 cells exposed to NaPT and ZnPT the cytoskeleton was damaged namely perforated possibly because of the fragmentation of cytoskeletal elements induced by the applied IC_{50}values of the agents. Results of TEM analyses indicated that the ultrastructure of the NaPT and ZnPT treated cells was altered. Microscopy results revealed the cytotoxic activity of NaPT and ZnPT on NIH/3T3 cells implying to induced cell death mechanisms, but the mode of action and the type of the cell death need to be further cleared.

WE144

Mixture toxicity of three NSAIDs and pharmaceutical wastewaters using Daphnia magna and a luminescent bacterium

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Pharmaceutical residues in water may cause serious ecological consequences. While pharmaceuticals in aquatic environment exist in mixture, their interactions and ecotoxicity are not well-characterized. Pharmaceutical industries and hospitals are among the major sources of release of pharmaceuticals to ambient environment. In the present study, three frequently used non-steroidal anti-inflammatory drugs (NSAIDs), i.e., diclofenac (DIF), ibuprofen (IBP), and naproxen (NPX), were chosen and their toxicities in mixture were evaluated. In addition, influent and effluent of wastewater treatment plants of two university hospitals in Korea were chosen. To determine ecotoxicity, a standard acute 48 hr *Daphnia magna* test and Microtox assay using *Allivibrio fischerii* were used. In addition, the heartbeat of *D. magna* measured following 1 hr exposure was also used as an observation endpoint. For each NSAID, a range of concentration (3, 10, 30, 100, and 300 mg/L) was tested. For mixture study, mixture ratios of DIF, IBP, and NPX were determined based on the ratio of each NSAID detected in the hospital wastewater. For the *D. magna* 1 hr heartbeat assay, 7-10 days old *D. magna*were exposed to a series of dilutions of test solutions in the 96-well plates. After 48 hr exposure to three subsets, immobility of *D. magna* was increased in a dose-dependent manner. In the *D. magna* heart rate test, NPX showed a decreased trend, while DIF and IBP showed an increased trend. After the exposure to NSAIDs mixture, heartbeat rate of *D. magna* was dose-dependently increased, but

that of wastewater was decreased. Decreased trends were observed in all Microtox tests. Due to similar modes of action of NSAIDs, predicted toxicities of the pharmaceutical mixture estimated based on concentration addition (CA) assumption showed similar levels of those measured directly from the mixture. Interestingly, one pharmaceutical wastewater sample among two collected samples showed greater toxicity in the effluent than the influent. Greater toxicity of the effluent can be partly explained by the effects of disinfection by-products and residual chemicals from the wastewater treatment processes. Ecotoxicity of pharmaceutical mixture dominated by NSAIDs appears to be reasonably estimated by concentration addition approach.

WE145
Novel ecotoxicology method based on metaproteomic analysis on soil
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Ecosystem assessment is turning the attention to the impact on the ecosystem function. Methodologies are not focused on a holistic understanding that could connect environmental function, impact and ecosystem services. Method in ecotoxicology evaluates the effects of stressors through a panel of model/sentinel species without the required global view. Proteomic-based methods that can assemble multivariate information have provided a global view of the cellular response to exposure but only from single species. Metaproteomics is a community analysis tool that could link the functional identity of proteins, from species directly responsible for ecosystem services, with the population eco-physiological response to stressors. We present our development of a metaproteomic-based method to track the dynamics of soil microbiome that could be translated into understanding the impact of pollutants on soil function. In the pilot study, natural standard soil LUFA 2.2 were exposed to copper (bulk and nanoparticles) in two concentrations (100 and 500 mg/Kg) for 28 days. Soil microbiome was analyzed by metaproteomics and soil biochemistry. Proteins were extracted from the soil and fractionated by LC. Peptides were digested and then analyzed on an Orbitrap Velos Pro. The MS/MS spectra were analyzed by MetaProteomeAnalyzer open source software. Our results indicated that the key factor in our method development was: i) the utilization of a standardized environmental sample and microbiota that could guarantee experimental robustness and reproducibility for metaproteomic data; ii) miniaturizing the exposure experiment to a platform that could be performed in any basic lab, and iii) implement protein extraction and sample fractionation. Regarding enzyme activity, for phosphatase, a significant decrease was detected for the highest concentration (500 mg/Kg nano Cu) while for glucosidase and urease the activity increased, being significantly for the concentration of 500 mg/Kg bulk Cu. Our metaproteomic data indicated that the complexity of soil microbial community could be correlated to both changes in the biodiversity and the molecular responses of the communities to exposure to pollutants and therefore interpret and predict the ecosystem response to identical stressors.

WE146
Physiological responses of Caenorhabditis elegans to arsenic tolerant Lysinibacillus sphaericus B1CDA.
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High concentrations of metals found in the environments pose serious ecological risk to living organisms. The first biota that undergoes direct and indirect negative impacts of heavy metals are the microorganisms. Metals not only affect microorganisms by reducing their number, diversity, biochemical activity but can also lead to establishment of metal tolerant microbial populations. Studies have reported effect of metal contamination on the physiology of metal tolerant microbes. However, their impact on the food chain and subsequent host has not been explored properly. *Caenorhabditis elegans*, a bacteriovorous nematode is naturally colonised with microorgnaisms in the environments, and this makes it an interesting model to study host physiology. *Lysinibacillus sphaericus* B1CDA, an arsenic accumulating strain isolated from an arsenic-contaminated site, has arsenic transforming ability (arsenate to arsenite) has been used in this study. The aim of the study was to investigate the nematode host physiology when fed the arsenic tolerant bacteria, *L. sphaericus* B1CDA,in the presence and absence of arsenic. *C. elegans* were fed bacteria exposed to both arsenate (AS_V)and arsenite (AS_{III}) and gene expression, lifespan and fat analysis were used to explore the physiological responses. Nematodes fed arsenic pre-treated *Escherichia. coli OP50* (control) and *L. sphaericus* B1CDA during the L1 to early adult (L4) stage showed altered gene expression in genes associated with metal stress, oxidative stress, innate immunity and fat metabolism. The altered fat metabolism of the nematodes was confirmed using fat staining. The worms fed with *L. sphaericus* showed extended lifespan compared to the *E. coli* control, however, the lifespan was significantly reduced in the presence of AS_V or AS_{III}. The lifespan reduction was more prominent in the presence of As_{III} than for As_V indicating the difference in toxicity of the two species of arsenic. A better understanding of the effects of metal tolerant bacteria on hosts can reveal possible mechanisms of host-microbiota interactions and their implications in stress response and diseases.

WE147
RELATIONSHIP BETWEEN POLLUTION AND HUMAN HEALTH
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RELATIONSHIP BETWEEN POLLUTION AND HUMAN HEALTH
Ecotoxicology enable us to understand the chemical effect (contaminants) on individual, population, natural communities and ecosystem. This study is essential in interaction of organisms with its environment. While, human toxicology include identification & assessment of environmental exposure of human health and pollutants, mechanism by which it get into human system, induce disease, adverse effect, prevent and estimation of acceptable level of exposure for protection of public safety. The safety of public health is paramount to study of the relationship between human being and environmental pollution. At this level, strict regulation of industrial discharge is therefore recommended. Omics can be applied in human toxicology for proper assessment of effect of pollutants on human health. It involves total data analysis in information gathering in a body, thus enabling independent experimental to be carried out (invivo) in such body, in order to contain the effect of the pollutants or find a solution to any disease caused by the pollutant.

WE148
Toxicity of Glyphosate and AMPA to Aquatic Bacteria
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Glyphosate [N-(phosphonomethyl)glycine] is the most frequently used active ingredient in broad-spectrum herbicide formulations. There is concern that glyphosate-based herbicides may adversely affect environmental microorganisms. In this study, six bacterial strains isolated from a lake ecosystem were examined for their susceptibility to glyphosate and the primary degradation product aminomethylphosphonic acid (AMPA). The toxic responses of the environmental bacteria were compared to responses in laboratory strains of *Escherichia coli*, *Pseudomonas fluorescens* and *Aliivibrio fischeri*. Minimum inhibitory concentration (MIC), regrowth potential and changes in enzyme activity were examined under different nutritional conditions in the presence and absence of AMPA or glyphosate. Traditional MIC values for glyphosate were high when environmental bacteria and laboratory strains were tested in nutrient rich medium containing amino acids but often much lower when examined in nutrient poor medium under amino acid limited conditions. The regrowth potential of the environmental bacteria was also strongly affected by nutritional conditions. EC10 and EC50 values for sensitive aquatic bacteria were more than 100 fold lower in the absence of amino acids compared to EC values measured in the presence of an exogenous amino acid source (0.04 mg/L and 0.06 mg/L, respectively). Similarly, the bacterial toxicity of AMPA was also greater under nutrient limited compared to nutrient rich conditions. Inhibition (EC50) estimated from changes in bacterial regrowth was comparable to inhibition (EC50) estimated from changes in bacterial hydrolysis of fluorescein diacetate (FDA) suggesting that both methods can be used to indicate AMPA and glyphosate toxicity. In contrast, the standard luminescent test organism *Aliivibrio fischeri* was much less responsive to AMPA and glyphosate exposure suggesting that this bacterium is not a good surrogate for measuring microbial toxicity of these chemicals. The results of the study corroborate studies suggesting that glyphosate may affect the biochemical pathway for aromatic amino acid synthesis in some environmental bacteria (Shikimate pathway). The results also suggest that nutrient limited aquatic bacteria may be adversely affected by glyphosate at environmentally relevant concentrations. The aquatic bacteria isolated in this study may be included in test batteries for toxicity screening of pesticides including glyphosate-based herbicides.

WE149
Using flow cytometry for bacterioplankton community analysis as a fast assessment tool complementary to Water Framework Directive in prioritization of impacted sites
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Freshwater ecosystems support an enormous variety of biological communities and provide simultaneously goods of critical importance to human societies. Anthropogenic disturbances such as mining, agriculture, industrial and household leachates, provoke losses in biodiversity through contamination, which affects the overall quality of water. In order to protect and ensure a good quality, Water Framework Directive (WFD) was implemented in Europe in 2000, however the WFD application is time-consuming and costly. This study proposes an assessment of the bacteriological community of the water column, using fast flow cytometry (FCM) methods and 16S rRNA gene-based denaturing gradient gel

electrophoresis (DGGE). The results of both bacterial community analysis techniques were used to compare with macroinvertebrate and diatom community structure analyses. Caima river (Northern Centre Portugal) was used as case study and 3 sampling sites were selected: site 1- with little impact; site 2- downstream a Waste Water Treatment Plant; site 3- exposed to mine drainage. Sites were studied over the 4 seasons of the year. In general, multivariate analysis performed for each biological community individually showed that all macroinvertebrates, benthic diatoms and bacterioplankton were able to clearly separate the sampling site burdened with excess of nutrients and turbidity (site 2) and the river headwater (site 1), associated with high oxygen levels. The DGGE analysis of the bacterioplankton community structure matched the results obtained by FCM. Therefore, FCM can be a rapid bioassessment tool to complement the WFD methodology, by signaling/prioritizing potentially impacted areas needing attention to reach the good ecological status demanded by WFD. However, further investigations are needed to confirm the feasibility of this method, as a complementary tool for water quality assessment.

WE150

Gut microbiome attenuates toxicity of cadmium in Caenorhabditis elegans

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Soil microorganisms carry out important role in soil ecosystem, such as, cycling of carbon and nutrients, support of plant growth, and fitness and stress response of soil organisms. In this study, we investigated effect of soil microbiome (SMB) on the response of the soil nematode Caenorhabditis elegans when exposed to one of the major soil contaminants, cadmium (Cd). As C.elegans is a bacterivore, soil microbiome was supplied as food source, which could also provide role of gut microbiome on the fitness of C.elegans, as well as stress response to chemical exposure. C.elegans was fed with microbiome sampled from Korean soil and life history traits and its stress response to Cd exposure were compared between SMB- and OP50-fed worms. It was found that SMB-fed worms showed faster growth rate, higher reproduction and longer lifespan, compared to OP50-fed worms. Increased reproduction by SMB feeding was maintained over 10 generations. After confirming beneficial effect of microbiome to C.elegans, we then investigated effect of SMB feeding on C.elegans stress response by using Cd exposure. Increased tolerance to Cd exposure was found in SMB-fed worms when using reproduction as endpoint. We postulated enhanced tolerance in SMB-fed worms may be due to boosted immunity by microbiome. To test this hypothesis, gene expression analysis was conducted using panel of immune (i.e. clec-67, irg-2, lys-1, lys-2, pmk-1) and chemical response genes (i.e. cdr-2, mtl-2, cyp35a2, ugt-1, sod-3). Clear difference was observed between immune and chemical stress response genes expression in SMB- and OP50-fed worms. Cd exposure led to increased expression of Cd biomarker gene (i.e. *mtl-2*) in OP50-fed worms, whereas such an activation was not observed in SMB-fed worms. On the other hand, expression of immunity related genes (i.e. *irg-2, clec-67, lys-2*) was more pronounced in SMB-fed worms than OP50-fed ones when exposed to Cd. Gene expression result insinuates that boosted immunity by microbiome might play a role in attenuated toxicity of subsequent Cd exposure. Finally, we investigated *C.elegans* gut microbiome by comparing microbial community in absence and presence of Cd. Proportion of Actinobacteria phylum, known as Cd resistant, significantly increased after Cd exposure. Our results suggest SMB affects response of *C.elegans* to Cd exposure, highlighting the importance of gut microbiome on host stress response mechanism.

Advances in Soil Ecotoxicology and Risk Assessment - Impact, Ecotoxicity Tests, and Concepts for a Retrospective Environmental Risk Assessment (P)

WE151

Cocoon hatching success as ecotoxicological endpoint: A potential tool to assess metal contaminated soils

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South Africa produces large amounts of solid mine waste, covering vast areas of land in the form of tailings dam facilities (TDF). Tailings material contains high levels of metals which poses potential risk for the environment and human health due to leaching and erosion into the surrounding area. Agricultural practices such as tilling and chemicals added also bring these soils into perspective regarding erosion and leaching. Both these soils are disturbed on a physical level, influencing the soil structure, chemical composition and soil fauna present. The aim of this study was to investigate the possibility of utilizing cocoon hatching success as ecotoxicological endpoint to assess metal contaminated soils. Ten *Eisenia andrei* cocoons were exposed to 250g of mine tailing soil, agricultural soil, natural soil and to the control in plastic containers (12 cm x 6 cm) with perforated lids. Once in the soil, the cocoons were given a 23-day hatching window and a further 17-day growth period (to allow worms to be of suitable sizes for handling and weighing) before monitoring of growth started. Results indicated that the mean number hatchlings per cocoon were significantly lower ($p< 0.05$) in metal contaminated mine soils compared to the control, agricultural and reference soils. The same trend was also observed when investigating the growth of the juveniles over time. It can be concluded that utilizing cocoon hatching success as ecotoxicological endpoint might be sensitive and could possibly be used in the assessment of metal polluted soils. **Keywords:** Tailings dam facilities; earthworms; ecotoxicity; cocoons

WE152

Sewage sludge and sewage sludge char soil application: effects on earthworms' growth and reproduction

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Sewage sludge production in the world is increasing and its management is a growing problem worldwide. Land application of sewage sludge in agriculture or short rotation energy forestry plantations is a preferred sewage sludge disposal (reuse) route in European Union. As sewage sludge is rich in essential plant nutrients (N, P), it can be applied as fertilizer to soils in order to improve and maintain productivity of soils. However, the use of sewage sludge may pose environmental risk as it has an extensive range of heavy metals and persistent organic pollutants that may accumulate in the soil, be transferred from soil to soil dwelling organisms and be incorporated into the food chain. In this study we have examined the effects of sewage sludge and sewage sludge pyrolysis char soil application to earthworms *Eisenia fetida*. Adult *E. fetida* were exposed to 25-100 t ha^{-1} of sewage sludge and sewage sludge char in soil for 8 weeks. The impact on earthworm survival, growth rate and reproduction was evaluated.

WE153

Soil risk assessment of the fragrance ingredient, Helvetolide®

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Alicyclic musks and macrocyclic musks are fragrances that were developed in the 1970's as alternatives to the nitromusks and polycyclic musks which were already controversial due to their persistence and bioaccumulation potential. The olfactory characteristics of musks - warm, sensual, animalic and natural - are of great value to perfumers and consumers and as such musks are added in cosmetics as well as in laundry products. Alicyclic musks and macrocyclic alternatives are mostly readily biodegradable and have been shown not to be bioaccumulative despite their relatively high log Kow values. However, some of the earlier developed alicyclic musks are only partially biodegradable according to screening biodegradation tests. We report here the environmental profile of Helvetolide® and focus on the soil risk assessment, whereby the chronic effects on soil microorganisms and worm have been tested. The inhibitory effects of Helvetolide® on nitrogen transformation activity of soil microorganisms was assessed following the OECD TG 216 resulting in a 28d-EC10 value of 161 mg/Kg dw. The effects of Helvetolide® on soil worm reproduction were assessed using *Eisenia andrei* following the OECD TG 222 up to 250 mg Helvetolide®/kg dry mass soil. Soil samples were taken at multiple times for chemical analyses of Helvetolide® and of the transformation product helvetol. Rapid loss of the Helvetolide® was observed within the first week of the test, while most of the helvetol was eliminated after several weeks. The 56d-EC10 value based on number of offsprings hatched from the cocoons was determined to be 86 mg/kg dw based on nominal concentration of Helvetolide®. These results are used to derive the PNEC soil and compared with the PNEC soil derived by using the equilibrium partitioning method. Finally the equation of PEC soil /PNEC soil ratio below 1 confirms that Helvetolide® can be used safely and does not pose any risk to soil organisms. In addition, we demonstrate that the degradation product, helvetol is less bioaccumulable and less toxic than the parent molecule Helvetolide®.

WE154

Bioaccumulation kinetics of conazole fungicides in earthworms

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Earthworms are one of the most important bioindicators in the terrestrial environment as well as valuable endpoints for assessing the bioaccumulative potential and bioavailability of soil pollutants. Earthworms interaction with soil occurred via exposure to contaminants present in the integument and in the gastrointestinal tract, thus providing two possible routes of exposure. The resulting body burden is determined by uptake, metabolism and excretion processes while these processes themselves are soil-, compound-, and time-dependent. As such, bioaccumulation can be considered a complex process where the role of all influential factors remains still relatively unclear. Therefore, in the present study bioaccumulation was investigated in a series of experiments in order to account for the role of compound characteristics, of soil properties, and of the exposure time on the bioaccumulation kinetics of conazole fungicides by earthworms. Conazole fungicides represent widely used pesticides and their residues may persist in soil into following growing season(s) and pose toxicity to non-target organisms. Thus, they represent relevant soil contaminants with potential negative environmental impacts. In the study, two approaches were used for determining the extent of bioaccumulation (i.e., the bioaccumulation factor (BAF)) and compared. First, concentrations of chemicals in earthworms and in soil wereplotted against time. From the data, the uptake rate constants and elimination rate constants were gained and used for BAF determination ("the dynamic approach"). Second, BAFs were calculated for fixed exposure periods ("the static approach"). The study provides an insight on compound-earthworm-soil interactions, on the bioaccumulation process itself, as well as on the bioaccumulation potential of potentially risky pesticides by non-target species.

WE155

Aporrectodea caliginosa: A long-term laboratory study with different untreated and Copper treated field soils

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Currently, *Eisenia fetida/andrei* is used as model organism for earthworms in the environmental risk assessment (ERA) of Plant Protection Products (PPP). However, *E. fetida/andrei* is an epigeic species and nearly absent in most agricultural soils. Moreover, it is unclear if its sensitivity is comparable to e.g. endogeic species like *Aporrectodea caliginosa* (EFSA 2016) which live preferably in the mineral soil layer. Data for a meaningful comparison between *Eisenia* and other earthworm species are limited and in case of chronic (reproductive) endpoints even not available. Therefore, results on long term laboratory studies with earthworm species other than *E. fetida/andrei* might help filling the gap and further, give option to use those in standard testing for a more realistic ERA. *Field caught Aporrectodea caliginosa* were tested in two uncontaminated natural grassland soils and two soils from the same grassland site where Copper had been applied three times per year at a nominal rate of 8 kg Cu/ha/year for the past 14 years. The soils were collected (top soil; soil depth of approx. 5 cm; including vegetation) at two different field sites in South-Western Germany. The soils were deep frozen, thawed and homogenized before usage. Four replicates were used for each soil, 5 worms were inserted per replicate. The total duration of the experiment was 112 days. Mortality and biomass development were assessed at 28-day intervals until day 112; the reproductive output (i.e. number of cocoons and number of juveniles) was assessed once at day 112.

WE156

Assessment of soil ecotoxicity derived from the application of sewage sludge with biological indicators of soil health (microorganisms, plants and invertebrates) for remediation option appraisal

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Industrial activity, together with a high human population density and scarcity of useful land, have contributed to the proliferation of dumps, landfills and waste disposal points at different urban, peri-urban and natural areas in the Basque Country. According to the Basque Country Landfill Inventory (2005), the total number of landfills in this region is 1,277, thus being one of the most important ground pollution sources (affecting water sources and air quality as well). Among these inventoried landfills, "Landfill 17" is located at 43°19'28.9"N 2°40'30.9"W. This landfill was employed as sewage sludge disposal point for, at least, two decades. Among the species more likely to be affected by such disposal, plants, microorganisms and soil invertebrates like earthworms must be emphasized due to their close contact to the soil matrix and, hence, soil pollutants. The aim of the present work was to assess the ecotoxicity exerted by pollutants present in the sewage sludge repeatedly spilled into Landfill 17 as a preliminary step to remediation option appraisal, considering phytoremediation, bioremediation and vermiremediation technologies. For ecotoxicological evaluation, and in order to assess impacts at different levels of biological complexity, different standardized tests (OECD-222 and OECD-207) and the Calcein-AM assay (cell viability) were carried out using earthworms. Earthworms were maintained for 7, 14 and 28 days in soil amended with the sewage sludge containing trace elements (Pb, Ni, Cd and Cr), PAHs (benzo(a)pyrene) and pesticides (dieldrin). At day 7 and 14, measurements of weight loss, mortality and coelomocyte viability (Calcein-AM assays) were obtained. Effects on earthworm reproduction were assessed after 56 days of exposure. In addition, germination and root elongation plant bioassays were carried out in order to quantify soil phytotoxicity and, pollutant phytobioavailability. To this purpose, bioassays with *Lactuca sativa* and *Allium cepa* were performed. Microbial soils properties and other edaphic parameters were also assessed. Different sewage-induced effects were observed, compared to control soil (OECD standard soil), at different levels of biological complexity, demonstrating the accuracy of the combination of earthworm, plant and microorganism tools for soil ecotoxicity assessment. Acknowledgements: Basque Gov (IT810-13; ITO18-16), CTM2017-87766-R, AGL 2015-64481-C2-1-R and AGL2016-76592-R from MINECO, PhytoSUDOE-SOE1/P5/E0189.

WE157

The bioavailability of metals in multi-contaminated mining and quarry soils (NW Spain)

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Abandoned mining and quarry areas are a source of potentially toxic elements (PTEs), through to lixiviates or transfer processes of bioavailable fractions from mining wastes and tailings. Previous works have been carried out to assess metals availability through single and sequential soil extractions, and soil to plant transfer of potentially toxic elements (bioaccumulation on native plants, seed germination and root elongation bioassays). However, ecotoxicological effects on soil biota were not evaluated for these soils. In this sense, earthworms (*Eisenia fetida*) were exposed for 28 days to two mining soils from a lead/zinc mine (up to 43, 6700 and 32000 mg kg^{-1} of Cd, Pb and Zn), and two quarry soils from an old serpentine quarry (up to 147, 2600 and 18000 mg kg^{-1} of Co, Cr and Ni). The effect of soil exposure to earthworms was assessed at the biochemical level (acetylcholinesterase, catalase, glutathione-s-transferase, lactate dehydrogenase and lipid peroxidation through TBARs content) and DNA damage (comets assay). The exposure of earthworms was confirmed by metals bioaccumulation in tissues. Results showed a highest bioaccumulation for Ni, Pb and Zn, although bioconcentration factor values never exceeded 1. Regarding biochemical activities, Ache was always significantly inhibited in earthworms exposed to all the contaminated soils, however no clear signs of oxidative stress and DNA damages were recorded. The results from the earthworm's exposure point for the low bioavailability of metals in the tested soils (below than 15% of total content).

WE158

Impact of diclosulam, paraquat, glyphosate, metsulfuron and its mixtures on growth and reproduction of Eisenia fetida.

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Non target organisms are reached by herbicides during its application on agricultural systems. The aim of this study was to assess the impact of 4 herbicides and 2 mixtures frequently used during fallow, on the growth and reproduction of *Eisenia fetida*. The mixtures of diclosulam+paraquat and glyphosate+metsulfuron, as well as the single herbicides, were tested using OECD (2004) modified protocol (5% of peat content and 24 hours of light). Two concentrations were assessed, the commercial recommended dose and the worst scenario was represented by a 10 times higher dose than the former. Five replicates per treatment were used, and data was analysed by ANOVA followed by a Tukey test. The analysed variables were relative growth rate (RGR), number of hatched and unhatched cocoons, number of juveniles, and hatching rate. Diclosulam low dose was the only treatment with positive RGR, and different from glyphosate low dose which had the most negative RGR. This variable did not show correlation with the reproductive ones. On the contrary, glyphosate showed a stimulus in reproduction, presenting more juveniles, hatched and total cocoons than the untreated control. On the other hand, diclosulam presented lower number of hatched cocoons and hatching rate, than the untreated control. Metsulfuron did also decrease hatching rate. Diclosulam and paraquat mixed behaved very similar in terms of reproduction to single diclosulam and differed from paraquat. The mixture presented a lower hatching rate, and a tendency ($p = 0.0759$) to have fewer hatched cocoons than single paraquat. The mixture of metsulfuron and glyphosate showed intermediate results of hatching rate and number of juveniles regarding the single herbicides. In contrast, considering hatched cocoons, it resembles to single glyphosate and differs from metsulfuron. Herbicides had different effects on *E.fetida* depending on dose, mixture and response variable considered. On these conditions, diclosulam at both doses, single or mixed, posed the higher risk for *E.fetida*, decreasing its reproductive rate. On the other side, glyphosate at the recommended dose promoted *Eisenia*'s reproduction, not accelerating it, but increasing their number of offspring. The results highlight the need to consider different biomarkers in ecotoxicity studies.

WE159

Ecotoxicity of roadside soils in Moscow: finding correlations between traffic-related contaminants and their ecotoxicological effects

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Road transport is responsible for 57-75% of total emissions in urban areas and tends to be the key source of environmental pollution in cities [WHO, 2006]. Roadside soils perform as polycontaminated systems of intense pollutant accumulation originating from motor vehicles: total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAH), heavy metals (HM), deicing salts (DS). The purpose was to find correlations between traffic-related contaminants in the roadside soils and their ecotoxicity across a typical highway (125,000 vehicles per day) in the city of Moscow, Russia. The topsoils (0-3 cm depth) were sampled perpendicular to the road at 1, 6, 10, 18 and 50 m distances from the roadbed in three replicates. TPH, PAH, HM in total and phyto-available forms, and DS were determined. A battery of soil-contacting organisms was tested: phytotoxicity of rye (*H. vulgare* L.) and garden cress (*L. sativum* L.); *E. foetida* earthworm growth rate and mortality; basal and substrate-induced respiration activity, nitrogen fixation and the denitrification activity of the soil microbial complex. To determine the possible risk to aquatic ecosystems, the algal toxicity test (*S. quadricauda*) was provided. Correlations between «chemical» data and intensity of «biological» effects were analyzed. Concentrations of most contaminants declined to the background values with distance from the road increase. However, the toxicity of roadside soils was obtained for all examined organisms within the whole 50 m zone. Live organisms exhibited different sensitivities to roadside soils pollution. The intensity of inhibition effects decreased in order: higher plants > earthworms and microorganisms > algae. The risk for aquatic ecosystems was assessed as low. Higher plants toxicity correlated with TPH, PAH, some HM, and DS; earthworm toxicity correlated with TPH, some PAH, HM, and DS; microorganism toxicity correlated with TPH and DS; algae had no observed correlations with contaminants. TPH and DS were general ecotoxicants affecting all organisms. Higher plants may be considered the PAH indicators and earthworms as HM indicators. Thus, biological methods are a prospective tool for assessing roadside soils. Chemical analysis must be accompanied by biological studies for comprehensive ecological assessment. A set of higher plants and earthworms may be recommended as the reduced test-battery of relevant organisms for cost-effective assessment of the toxicity of roadside soils.

WE160

Toxicity of lanthanides to boreal soil invertebrates over time

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The advent of new commercial technology applications has led to recent exploratory efforts within Canada for future mining of lanthanide rare earth elements (REE). Although naturally present in soils, additional inputs occur as a result of mining and refining operations, leachate from landfill due to discarded technology, and the application of phosphorous fertilizers (produced in part by REE rich monazite minerals). As such, certain REEs have been identified as priorities for chemical risk assessment under the Government of Canada's Chemicals Management Plan. Data gaps exist for soil hazard data in Canadian boreal regions, where new mining activities are likely to occur. As a result, a series of studies were conducted to assess the toxicity of Praseodymium (Pr), Samarium (Sm), Neodymium (Nd) and Yttrium (Y) in two horizons of a brunisolic boreal forest soil: an LFH and Ah-Aej-Bm horizon. The soils were spiked with metal salts, aged for 2 weeks, and then leached with deionized water to remove the excess salts. Three species of soil invertebrates (earthworm *Dendrodrilus rubidus*, springtail *Proisotoma minuta*, and oribatid mite *Oppia nitens*) were exposed to a series of concentrations of the leached soils. Of the REEs assessed, the two most toxic elements were aged for 6 months and toxicity reassessed. Toxicity varied with species, with Pr being among the most toxic REEs assessed to date. The toxicity data will be presented, along with discussion of the implications of metal availability across horizons and time.

WE161

Toxicokinetics and toxicodynamics of cadmium in soil collembolan Paronychiurus kimi exposed in simplified soil solutions

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Conventional toxicity testing has proceeded in a manner that assesses the toxicity of a chemical at a given point in time, which ignores the effect of time on uptake and toxicity of a chemical. In this study, time-dependent accumulation and toxicity of cadmium in soil collembolan *Paronychiurus kimi* were investigated with the aim to link toxicokinetics to toxicodynamics. The *P. kimi* were exposed for 10 d to different cadmium concentrations (uptake phase) in simplified soil solutions embedded in quartz sand, followed by a 14-d elimination phase in clean simplified soil solutions embedded in quartz sand. Internal Cd concentration and mortality were determined at 7 different time intervals. Internal Cd concentration in *P. kimi* increased with increasing exposure time and median lethal concentration (LC50) decreased with increasing exposure time. The overall results were interpreted from the perspective of toxicokinetics and toxicodynamics using a one-compartment model to calculate the uptake rate constant (k1) and elimination rate constant ($k_{2\text{-TK}}$). The results of present studies emphasize the need to reflect changes in toxicity values over time, which should be taken into acount in future toxicity testing and ecological risk assessment.

WE162

Assessing the effects of plant protection products on enchytraeids under field conditions: test of carbendazim as a toxic reference

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According to the Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms (EFSA PPR Panel, 2017), Specific Protection Goal options have been identified for different groups of soil organisms. For Enchytraeidae, "*the maximum initial magnitude of effect that might be tolerated in-field without impairing the general protection goal is suggested to be small effects less than 35% for months on the ecological entity 'populations of different enchytraeid species' or medium effects less than 65% for weeks.*" However, to validate magnitudes of effects on enchytraeids under field conditions, no active substance for the negative control is clearly identified. Our study aimed at testing under field conditions the effects of carbendazim - a fungicide already used as a toxic reference in earthworm field studies - on enchytraeids. The field study was conducted in a meadow located near Brensbach (Germany) in 2018. Eight study plots (4 control plots and 4 plots treated once with carbendazim at 10 kg a.s./ha in March 2018) with a size of 10 m x 10 m each were randomly located. Seven months after carbendazim application (in November 2018), six cores (10 cm depth, 5 cm in diameter) were taken using a split corer from a 6 m x 6 m core area in the central part of each study plot. Sampling, heat-gradient extraction and counting of Enchytraeidae were conducted in accordance with the ISO norm 23611-3 (2018). Our first results showed that enchytraeid abundance was significantly ($p < 0.001$, *t*-test, n=4) reduced by 72% in the carbendazim treatment compared to the control with a mean abundance of 21 (± 6, standard deviation) and 75 (± 15) individuals, respectively. These results are in accordance with a field study conducted in a grassland (Flörsheim, Germany) where the enchytraeid abundance was lower eight and sixteen weeks after single carbendazim applications (i.e. 9.72, 29 and 87.48 kg a.s/ha) compared to the control plots (Moser et al., 2004). Carbendazim could thus be considered as a relevant active substance for toxic reference in enchytraeid studies. Moreover, enchytraeid field studies could be adapted from the ISO standard method 11268-3 (2014) for earthworm field studies. Investigation of enchytraeid community composition (i.e. genus/species levels or r/K strategy types) would also bring key information on community change, biodiversity loss and potential impact on the soil functioning in agroecosystems.

WE163

A mesocosm experiment on the effects of nitric acid and sulfurc acid on below- and above-ground species: white radish and a Collembolan species

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Storage and transport of large quantities of chemicals have increased, resulting in an increase of chemical accidents. Since released chemicals caused by chemical accidents can cause not only widespread but also persistent damage on an ecosystem, understanding the effects of chemicals that can cause chemical accident has been of concern. We conducted a mesocosm experiment that allowed us to examine the effects of nitric acid and sulfuric acid on the soil organisms. The soil used in this experiment was sampled except for the organic layer from Deokso (N 37° 34' 56", E 127° 14' 8") and in October 2018. After defaunation, 2 kg of soil was transferred to each pot. We planted the mesocosm with white radish (*Raphanus sativus*), which is frequently cultivated in Korea. After 21 days, nitric acid and sulfuric acid at NOEC calculated by laboratory experiments was spiked and then, 30 adult individuals of Collembolan species (*Paronychiurus kimi*) native to Korea were inoculated. After 28 days, the mortality and reproduction rate of *P. kimi* was observed and root and shoot biomass of *R. sativus* were recorded. Unlike the results of laboratory experiments, root and shoot biomass of *R. sativus* was increased by the exposure of nitric acid and decreased by the exposure of sulfuric acid. The number of juveniles produced by adults *P. kimi* and survived adults *P. kimi* were decreased as the concentration-dependent manner in both nitric acid and sulfuric acid. These results suggest that low concentrations of chemicals released by chemical accidents can cause detrimental effects on the soil ecosystem.

WE164

Thirty Nine Threshold Guide Values (TGV-ex situ) to environmental risk assessment of contaminated soils with Cantareus aspersus

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The environmental risk assessment (ERA) of polluted fields and soils should ideally be carried out with complementary approaches (chemical and biological) conducted *in situ* and *ex situ*. If biological methods based on effect and bioaccumulation exist for bioindicators of the soil fauna such as landsnails, the methodology is currently only applicable on site to metallic elements (ME). To better understand the role of soil in snail exposure and also to give new relevant tool for polluted field managers, the aim of this work is to determine *ex situ* (under simplified exposure conditions) threshold guide values (TGV-$_{ex\ situ}$) in the viscera of the terrestrial snails (*Cantareus aspersus*) for 16 metallic elements (ME), 16 polycyclic aromatic hydrocarbons (PAH) and 7 polychlorinated biphenyls that are environmental indicators (PCBi). In this purpose, snails were exposed under controlled conditions for one month to various European soils (n=67). The soils chosen have different types and level of contaminations, and physico-chemical characteristics (granulometry, pH, organic matter content, carbonates, exchangeable cations, cation exchange capacity) that can influence the bioavailability of contaminants. After exposure, contaminants were measured in viscera by ICP/MS for ME and GC/MS for PAH and PCBi. Thirty nine TGV-$_{ex\ situ}$ were calculated for ME (As, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Tl, Zn), PAH (ACE, ACY, ANT, BaANT, BaPYR, BbFLT, BghiPL, BkFLT, CHY, dBahANT, FLT, FLU, IcdPYR, NAP, PHE, PYR) and PCBi (28, 52, 101, 118, 138, 153, 180). TGV-$_{ex\ situ}$make it possible to characterize *ex situ* excesses of transfer to the snails of bioavailable contaminants contained in contaminated soils by calculation of accumulation quotient (AQ) and sum of excess transfer (SET) index. The TGV-$_{ex\ situ}$ also permit to Evaluate the Risk of the Transferred ME, or PAH, or PCB by weighting AQ and SET by the intrinsic toxicity of the contaminants based on the toxicity points. The TGV-$_{ex\ situ}$ of snails are a basis for interpreting contamination levels in the aim to help polluted soils managers in their choices: when TGV-$_{ex\ situ}$ are exceeded (AQ>1), the pollutant is bioavailable and therefore the polluted soil should not be used *e.g.,* for embankment after excavation. The presented results provide complementary solution to prioritize management of polluted soils (rehabilitation, monitoring) according to environmental risks that are estimated with risk index.

WE165

Ecotoxicological risk assessment of highly purified paraffin oils in banana plantations of Martinique and Guadeloupe.

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Paraffin oils of high purity (CAS 64742-46-7, CAS 72623-86-0 and CAS 97862-82-3) as produced by TOTAL Fluides are authorized pesticides in EU but are also used as an adjuvant for fungicides in banana plantations at an application rate of 15 L/ha equivalent to 12 kg/ha with maximum 15 applications. In the framework of Yellow and Black Sigatoka disease control caused by *Pseudocercospora musae (formerly Mycosphaerella musicola) and Pseudocercospora fijiensis (formerly Mycosphaerella fijiensis*), the fungicide mixture is applied locally in order to protect the youngest leaves of the banana tree. The efficacy of paraffin oils is based on physical effects by making a film that prevent organisms from breathing. Such process gives its insecticidal and acaricidal properties. That is why when used as an adjuvant for fungicide, particular attention was given to the risk to terrestrial arthropods following the treatment of Black and Yellow Sigatoka in banana. The risk assessment conducted for terrestrial arthropods including pollinators and soil arthropods, concluded to an acceptable risk for all groups except for the non-target arthropod representative species *Typhlodromus pyri* under EU standard risk assessment scheme. The toxicity reported for the phytoseid mite *Typhlodromus pyri* together with the high application rate and the high number of applications lead to a potential risk conclusion. Therefore, a risk assessment refinement was conducted, starting with taking into account the particular agricultural practices of banana plantations where banana trees are trimmed on a fortnight basis, regularly eliminating the oldest leaves. Consequently, the actual number of applications received by each leaf is drastically reduced and limited to a maximum of 2 applications under the growth conditions in Martinique and Guadeloupe. On the other hand, the ecology of the phytoseid mites shows that they live mainly under the leaves, and are therefore less exposed to the sprayed mixture which spraying is “crop-directed” and reaches mainly the upper face of the leaves in the canopy. Moreover, although it is the most sensitive taxonomic group to paraffin oils, phytoseid mites are not ecologically important in banana plantations compared with other tropical crops where they are at least 10 times more numerous. Based on the refined exposure, no unacceptable risk was found for non-target arthropods from the use of Paraffin oils in bananas at the recommended rate.

WE166

The sensitivity of Caenorhabditis elegans (Nematoda) to pesticides in soil compared to other standard test organisms

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Nematodes are, due to the evolution of different feeding strategies (bacterial-feeders, fungal feeders, ominvors, predators), key drivers for ecosystem services such as nutrient cycling, which is also recognized by EFSA (EFSA Pannel on PPP and their residues, 2017, EFSA Journal 15 (2), 4690). *Caenorhabditis elegans* is a known representative of soil nematodes and can be used as a test organism for assessing the toxicity of plant protection products (PPP). The well-established soil toxicity test according to ISO 10872 allows setting up chronic toxicity endpoints for PPP in soils on the basis of a quick (96h) and inexpensive test system. However, until now, only few data are available to compare the sensitivity of *C. elegans*compared to the standard soil test organisms as required by regulation (EC) 1107/2009. Therefore, we tested 13 PPPs (4 fungicides, 4 insecticides, 4 herbicides, 1 nematicide) in terms of their toxicity on *C. elegans*’ reproduction and growth in spiked soil (Lufa St. 2.2). We provide a comparison of nematode toxicity to available endpoints of the standard soil test organisms earthworms, springtails and soil mites based on most recent publicly available EU documents.

WE168

Evaluation and validation of field studies with soil organisms exposed to Plant Protection Products

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The higher tier risk assessment for non-target soil meso- and macrofauna (e.g. earthworms, collembolan and mites) exposed to Plant Protection Products (PPP) is currently mostly based on studies in the field. Recommendations dealing with the study setup, the conduction of field studies with earthworms as well as the requirements of the test site are currently captured in the guidance ISO 12268-3 (2014 [1]). Guidance on how to evaluate the performance of field studies with earthworms is given in De Jong et al (2006) [2]. For other soil organisms (e.g. collembola or mites), no guideline or guidance is available so far. From a regulatory point of view, several requirements should be considered in terms of determination of captured animals, evaluation of test results as well as choice of statistical evaluation procedures and interpretation of test results. Especially the current statistical power of the field tests with soil animals is a matter of high concern. Due to the high natural variability and the current set up of field studies (e.g. 4 replicate samples in each of 4 replicate plots), the statistical detection of results at the desired level can be hampered. In order to avoid masking ecologically relevant effects, the test design as well as the way of interpreting the results should be adapted. First results of an ongoing research project tackling several of these points have already been presented [3] and will finally lead to proposal for an OECD Guidance for earthworm field studies. In this contribution, we aim at proposing a way forward in the evaluation of currently submitted field studies with earthworms and soil mesofauna, including a quality check, the possible setting of ecological relevant effects and an interim proposal for assessing the study results in view of the planned revision of the Guidance Document on Terrestrial Ecotoxicology by EFSA. [1] ISO. International Standord Organisation (2014): ISO 11268-3. Soil quality – effects of pollutants on earthworms. ISBN 978 0 580 79961 7 [2] De Jong et al (2006): Guidance for summarizing earthworm field studies. RIVM Report number 601506006/2006. ISBN 90-6960-254-0 [3] Daniels et al (2018): The application of the CPCAT approach reduces shortcommings of effect detection for earthworm field studies. SETAC Europe annual meeting, Rome [PS1]Reference einfügen (vom anderen Abstract [PS2]Reference einfügen [PS3]Poster Daniel als referenz einfügen Haben wir bisher nichts weiteres?

WE170

Sensitivity comparison of double- and triple- bioluminescent enzyme systems to soil toxicants

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Intensive production and use of new chemicals in agriculture lead to an extreme soil contamination. Modern methods for assessment of quality and contamination of the soil, for example, methods of chemical analysis, are not always able to quickly predict the negative impact of the new used chemicals on humans, ecosystems and the biosphere as a whole. In this paper, we demonstrate a new principle of biochemical design for the development of a new generation of enzymatic bioassays. The principle is based on the replacement of living organisms to sets of enzymatic biotests, which are gathered together with the following feature - “key enzymes represent functioning of a body”. The basis for the design of both specific and integral methods of analysis is the coupled enzyme system of luminous bacteria NADH: FMN-oxidoreductase (R) + luciferase (L), which proved itself earlier as a simple and low time-consuming tool in the field of the bioassay. The unique feature of R + L enzyme system is the possibility of coupling with R + L enzyme system different NAD^+-dependent enzymes. In this case, there is a perfect tool for measuring the activity of more than 100 enzymes. In addition, the use of enzyme reactions as a test system instead of living organisms allows manipulating the sensitivity of creating enzymatic bioassays, depending on the problem to be solved. The aim of this work was to compare the sensitivity of double- (R + L) and triple- (LDH + R + L) enzyme systems to pesticides and metal ions for their future application in environmental monitoring of soils. The study of the sensitivity of the enzyme systems to aqueous solutions of toxicants (malation, diazinon, and copper chloride (II), shows that the triple enzyme system (LDH + R + L) has greater sensitivity, from 3 to 15 times to the toxicants than R + L enzyme system. The obtained values of the EC_{20} parameter, which shows the detection limit of the enzyme systems to the toxicants, show that the sensitivity of the enzyme systems to the toxicants depends as on the nature of the toxicant as on the soil texture. For example, the sensitivity of R + L enzyme system to copper chloride (II) is 1-fold decrease when the chernozem soil sample has contaminated this toxicant. In contrast, the sensitivity of LDH + R + L enzyme system to copper chloride (II) is 3-fold decrease when the medium loam soil sample has contaminated the toxicant. The received results show huge abilities of the enzyme systems for the assessment of contamination of the soil samples.

WE171

Tracing the metabolism of 2,4-D in Cupriavidus necator JMP 134 using deuterium and carbon isotope probing

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Pesticides are organic contaminants of particular interest since they are applied deliberately and in large amounts to soils. The main pathway of their ultimate detoxification is considered to be microbial degradation. The carbon or nitrogen from an organic contaminant can be used by microorganisms to synthesize their biomass compounds. After the microorganisms die, these biomolecules are incorporated into the organic matter of soil or sediment forming ultimately the so-called biogenic non-extractable residues (bioNER). Stable isotope tracers such as ^{13}C or ^{15}N enable the detailed study and quantitation of the C and/or N flux from a targeted pesticide via microbial biomass to bioNER. However, ^{13}C or ^{15}N-labelled pesticides are not easily available; they need to be custom synthesized and at high costs. In contrast, deuterated compounds are cheaper and available more easily, since they are commonly used as internal standards to compensate matrix effects in quantitative analyses by LC-MS/MS. Here we test the applicability of D labeling to study the fate of pesticides with a particular focus on D incorporation into the microbial biomass. A model bacterium *Cupriavidus necator* JMP 134 was grown on D and ^{13}C labeled 2,4-Dichlorophenoxyacetic acid (2,4-D) -a widely used herbicide- and the turnover mass balance was determined for both labeling approaches. The extent of D incorporation into the microbial biomass and into phospholipid fatty acids of *C. necator* was quantified and compared with that of ^{13}C. If deuterium labeling proved successful, it would be a cheaper and more realistic approach to study the fate of pesticides.

WE172

Nanoremediation: evaluation of effectiveness for heavy metal removal and ecotoxicological impact

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Among the risks that threaten the soil/water system maintenance, the heavy metal (HM) contamination in soil is of great concern due to its numerous sources, toxicity, non-biodegradability and accumulative behavior. The need of an efficient *in situ* stabilization strategy to ensure a reduction on metal and metalloids bioaccesibility is still a goal to be achieved. Among the proposed remediation technologies, the zero-valent iron nanoparticles (nZVI) represents a promising option for its use in highly damaged environments to minimize bioavailable/mobile fractions of HMs in soil and water (nanoremediation). However, the environmental associated risks to the application of nZVI remain uncertain and research on the safety and ecotoxicity of nZVI is of extreme importance. In this study, we addressed the efficiency of the nanoremediation strategy in case of co-mingled HM pollution in a highly damaged soil environment. The objectives included monitoring the stability of the strategy for the *in situ* removal of Pb, Zn and Cd from a contaminated soil considered in the long-term, as well as its impact on different soil organisms. We applied a combined set of physical-chemical, toxicological and molecular analyses to monitor the remediation process. The integrative approach applied, using combined technologies and high-throughput –omics tools, enabled us to evaluate if the reduced bioavailability of HMs after nZVI treatment is equated with reduced environmental risk, and if it is demonstrated to be long term. The obtained results indicated that addition of nZVI successfully stabilized Zn, and particularly Pb, even at extremely high concentrations, and the efficient immobilization of HMs was reflected in a diminution of toxicity towards *Vibrio fisheri*. However, the extent and duration of the potential beneficial effects of this treatment were lower than few weeks. Moreover, our results indicated that no significant recovery of the microbial community structure, diversity and functionality was found in the nZVI-treated soil. Therefore, the lack of recovery of biodiversity and the reversible nature of nanoremediation must be carefully considered to validate this technology where assurance of medium to long-term immobilization of HMs is required. Keywords: nZVI, heavy metal, ecotoxicity, microbial community

WE173

EFFECTS AMETRYN MUTAGENIC FOR TRADESCANTIA PALLIDA

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Due to the increase in the appearance of contaminants in the environment and the search for an understanding of their effects on biota, environmental assessments at molecular levels are necessary, such as the quantification of the appearance of micronucleus during the cell division process, to monitor the mutagenic effects of the contaminant over the exposure time of the test species. The plant species *Tradescantia pallida*, is widely used in environmental biomonitoring studies, being sensitive for the detection of mutagenic compounds present in the environment, through the quantification of the appearance of micronuclei. Micronucleus are the result of chromosomal fragments that disintegrated during the process of cell division, resulting from the mutagenic action of the contaminant, which are not included in the nucleus of a new cell to be formed, being found in the cytoplasm. One of the environmental contaminants already detected in surface water and groundwater in some parts of the world and still used in some countries like Brazil, is the herbicide ametryn. This contaminant is applied to sugarcane crops, having toxic effects on the microbial community and other non-target organisms. Thus, this work aimed to evaluate the mutagenic effects of ametryn (125 µg/mL) for *T. pallida*, in soil with no herbicide application history. It was also evaluated the influence of microbial biostimulation in the presence of the contaminant, through the application of Tween 80 surfactant, as well as of the bioaumentation with the addition of microbial consortium adapted to the presence of ametryn. The effects evaluated was the quantification of the appearance of micronuclei in the treatments. As a positive control, trifluralin and negative control deionized water were used. Ametryn was found to cause mutagenic effects on *T. pallida* in soil, resulting in a higher frequency of micronucleus (5 MCN/300 tetrad). However, with the application of surfactant and microbial consortium the mutagenic effect (1 MCN/300 tetrad), resembling the control soil, which had no application of ametryn. Therefore, such responses are of concern because they cause damage to germ cells and somatic cells, which can result in hereditary diseases. Thus, the necessity of the evaluation of the mutagenic properties for organic compounds, becomes more and more essential to employ measures for the bioremediation of contaminated areas.

WE174

Species-specific impact of silver-graphene oxide on seed germination

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Silver-graphene oxide (Ag–GO) is widely applied in environmental fields as antibacterial agents, catalysts, and absorbents due to its unique properties. The potential for using Ag–GO to control agricultural diseases is also increasing, and this may lead to its release into the terrestrial ecosystems. However, little is known about the impact of Ag–GO on plants. In this study, we determined the impact of Ag–GO on seed germination and early growth of crop species by analyzing the germination rate, growth of roots and shoots, hydroperoxide (H_2O_2) accumulation, and the uptake of Ag in alfalfa, radish, and cucumber treated with 0.2–1.6 mg ml^{-1} of Ag-GO. Treatment with Ag–GO at 0.2 – 1.6 mg ml^{-1} increased the shoot growth of radish but decreased that of cucumber at 0.4 mg ml^{-1}. In addition, 0.2 mg ml^{-1} Ag–GO enhanced root elongation of radish but inhibited that of alfalfa treated with 0.2, 0.8 and 1.6 mg Ag-GO ml^{-1}. Ag–GO treatment induced H_2O_2 in alfalfa and radish in a concentration-dependent manner. The amount of Ag accumulated in seedlings under Ag–GO treatment was concentration-dependent. Our study shows that Ag–GO may have negative effects on early growth of plants in a species-specific manner, and further studies employing a broad range of plant species are required to confirm its safety in its use as control agent of crops and other environmental fields

WE175

Pollution characteristics and health risk assessment of heavy metals in the vegetable collected from northern part of Serbia

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The objective of this study was to investigate heavy element contamination in three major vegetables (potato, carrot and onion) and determine the health risk of Serbian population in the agricultural region of the northern part of Serbia after the flood. Additionally, the content of 9 heavy elements (As, Cd, Pb, Cr, Cu, Co, Ni, Fe and Mn) in the flooded arable soil determined to evaluate the levels of heavy element pollution and human health risks using several indexes as ecological risk factor (Er), geo-accumulation index (I_{geo}), potential ecological risk index (RI) and pollution load index (PLI). These indexes pointed out different level of pollution of analysed soil with selected heavy elements. Furthermore, the potential health risk was assessed through target hazard quotation (THQ) for each element determined in vegetables as the ratio of determined dose of contaminant to a respective dose level. Also, the individual THQs together with total THQs (i.e. TTHQ as sum of THQs assessed for each crop) and hazard index (HI expressed as combined non-carcinogenic effects of multiple elements) were calculated. The THQ values for all analysed elements were below 1, indicating that intake of a single element through consumption of vegetables do not pose a significant potential health hazard. Potential health risk evaluated as HI was < 1, not representing significant risk to Serbian adult population. To the best of the our knowledge, this is first investigation carried out in the flooded arable region (of Vojvodina Province) as attempt to assess health risk which might be attributed to chain soil-plant-human.

WE176
Density-dependent growth responses of Arabidopsis to copper: High densities are beneficial for efficient contaminant uptake
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Plant growth responses to toxic copper concentrations are density-dependent. Copper toxicity is reduced at high plant densities for reasons that are not clear. One possible mechanism is the release of citrate from roots in response to excess copper. Citrate binds metals, and thus pooled exudates at high plant densities could reduce copper uptake. This hypothesis was tested using *Arabidopsis thaliana* cultivars that either produce (wild type, Columbian-0) or do not produce (mutants m65 and m85) citrate in response to metals in soil. An ecotype reported to be tolerant to metals (Santa Clara) was included for comparison. Copper treatments were applied five days after transplanting seedlings at rates of 75, 125, and 175 micrograms copper per gram of soil. Plants were grown at four densities (1, 3, 5, and 7 plant per pot). Plants were harvested before bolting, 15 days after treatment. Dry shoot biomass was measured, and plant material was then digested in nitric acid and copper content analyzed by atomic absorption. Copper uptake was more efficient at higher plant densities, indicating that resource competition enhances copper extraction. For each cultivar, the amount of copper removed from the soil increased with plant density. At each density, the Santa Clara ecotype was the most efficient in copper extraction. Copper toxicity was reduced at higher plant densities, but the role of citrate in reducing toxicity is not clear. For both of the mutant, non-citrate producing genotypes, the concentrations of copper per gram biomass at harvest were more than double those of wild type plants. This supports the hypothesis that root exudation of citrate reduces copper uptake on a per biomass basis. However, the greater copper uptake by the mutant varieties did not result in greater toxicity in comparison to control plants. Copper treatments increased variability of plant size, and this effect increased with copper dose. These findings are directly relevant to phytoremediation of soils and to standard ecotoxicological testing, which typically does not take into account the importance of plant densities. More research must be done to understand the mechanism behind density-dependent effects. The impact of copper on size variation among plants may also have important consequences for competition outcomes in contaminated soils.

WE177
Influence of soil physicochemical properties on the depth profiles of perfluoroalkylated acids (PFAAs) in soil along a distance gradient from a fluorochemical plant
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Perfluoroalkylated acids (PFAAs) have been produced for over five decades. Their hydrophobic and lipophobic character makes them suitable for a wide range of applications. PFAAs may enter the environment where they accumulate and may cause detrimental effects. The widespread use of PFAAs has led to a global presence. As a result, the major global manufacturer, 3M, phased-out the production of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in 2002. Despite these regulatory measures, these compounds can still be detected in high concentrations in the environment and biota. The fluorochemical plant near Antwerp has been characterized as a PFAAs hotspot of environmental contamination. In the present study, we investigated how 15 PFAAs (11 perfluoroalkyl carboxylic acids (PFCAs) and 4 perfluoroalkyl sulfonic acids (PFSAs)) were distributed in different soil layers collected at three sites representing a distance gradient from a fluorochemical plant. Additionally, we examined correlations between multiple physicochemical properties of the soil (temperature, pH, clay content and total organic carbon (TOC)) and the PFAA concentrations in the soil layers. Finally, we tested the correlations between PFAA concentrations and microbial activity, microbial biomass and soil respiration in the top layer at five sites, along the same gradient. The only compounds that were detected in more than 50% of the soil samples were PFOA and PFOS. In the top layer, PFOS concentrations were positively correlated with pH and increased up to 2km away from the plant. Hereafter concentrations decreased. Similarly, PFOA concentrations were also positively correlated with pH and decreased at sites further than 2km away from the plant site. Soil temperature was not correlated with concentrations of PFOA or PFOS in the top layer. Soil respiration was positively correlated with PFOS concentrations, whereas microbial activity was not correlated with both PFOS and PFOA concentrations. The outcome of this study will provide more information on the factors that might affect sorption of PFAAs to soils and the potential effects of PFAAs on microbial communities in soil.

WE178
Implementation of bioavailability in the derivation of soil threshold concentrations for arsenic: linking ecotoxicological data with soil extractable metal concentrations and soil properties
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Soil quality thresholds expressed as single total metal concentrations do not reflect the large variation in bioavailability of these metals across soils. A realistic risk assessment of metals in soil however should consider such bioavailability aspects. Therefore, the aim of this project is to connect bioavailable fractions of arsenic with ecotoxicological effect concentrations, taking into account soil properties (texture, pH, organic matter content etc) and various metal extractions (1M NH_4NO_3, 0.01M $CaCl_2$, $Ca(NO_3)_2$with ionic strength corresponding to soil solution, DTPA/$CaCl_2$, 0.43M HNO_3, aqua regia). Arsenic was chosen due to its high relevance as a soil contaminant, its low data availability compared to other metals and because it is an element of concern included in many soil regulations. Six soils covering a wide range of Central European soil properties were chosen and spiked with sodium arsenate dibasic heptahydrate (Na_2HAsO_4•$7H_2O$). Chronic toxicity endpoints were tested for several plants, invertebrates and microbial endpoints, according to ISO standard guidelines, allowing derivation of soil threshold concentrations via a species sensitivity distribution (SSD) approach. The ecotoxicity results (given as NOEC, $EC_{10}$and EC_{50}values) for the endpoints tested based on nominal concentrations, as confirmed by total (i.e. aqua regia extractable) concentrations, vary up to 2 orders of magnitude among the soils tested. The impact of soil properties and the variation in ecotoxicity among soils was however strongly dependent on the species and endpoint tested. The extraction strength of the different methods and soils differ at least by an order of magnitude and the order for most soils is NH_4NO_3< $CaCl_2$< $Ca(NO_3)_2$< DTPA < $HNO_3$and aqua regia. Expressing the ecotoxicological data based on the different extraction methods significantly affects the remaining variation in ecotoxicity results among soils and can decrease this variation among the soils tested to a factor of minimum 2 to 5. The best extraction method, i.e. resulting in least variation in toxicity data among soils, differs for the different species and endpoints tested. It is currently investigated to what extent the variation in soil properties can explain the remaining variation in toxicity data based on the different extraction methods. All this information will be combined and used to include bioavailability into the derivation of soil threshold concentrations for arsenic.

WE179
Removal of arsenic from natural waters by membrane technologies and adsorption
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Arsenic contamination in agricultural soils and freshwaters is a serious and global threat to food production and safe water supply. High arsenic concentrations have been found in agricultural soils e.g. at chemical weapons destruction areas of the World War I in France and the old mining regions in Germany in Europe. Consequently, high loads of As and other pollutants are released to water systems from the remains of weapon destruction and former mining activities. To avoid pollution of adjacent areas, the leachate waters have to be collected and treated. To ensure food and fodder safety, the agricultural use of those areas requires particularly economically and environmentally sustainable treatment processes.\nThe aim of this research was to remove arsenic and other contaminants efficiently from polluted surface waters with membrane and adsorption technologies, and to evaluate the sustainability of these processes. First, arsenic removal by low-pressure reverse osmosis and nanofiltration was studied in screening experiments. Synthetic waters similar to the contaminated natural waters in the pilot areas in France and Germany were used and the effect of pressure and temperature was studied to determine the optimal conditions for arsenic removal. In addition, the removal of other contaminants was studied. The results show that membrane technologies are suitable purification methods for the synthetic waters and thus potential purification methods for the contaminated waters of the pilot areas.\nAfter the screening experiments, arsenic removal from real contaminated natural waters originating from the pilot area in France was performed. The experiments were conducted separately and sequentially by

membrane and adsorption technologies. A low cost schwertmannite based adsorbent prepared from side products was utilised in these experiments and compared with a commercial adsorbent. The results from the purification tests show that arsenic removal of the schwertmannite adsorbent was superior to that of the commercial one. Benefit from combining membrane technologies and adsorption into a hybrid process was also evaluated. The sustainability of the purification processes was evaluated based on data obtained from the experiments and literature. The ultimate aim of this research is to develop a sustainable arsenic removal process taking into account environmental, economic and social aspects.

WE180

What's in our Playgrounds? Distribution and Bioaccessibility of Metals in Urban and Peri-Urban Parks in Metro Vancouver, BC, Canada

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Metals can be introduced into urban soils including parks and playgrounds through natural processes, automobile exhaust and tire wear, metal fabrication and recycling industries and the use of inorganic fertilizers and pesticides. Exposure to metal contaminants in the soils can occur during use of parks and playgrounds through soil contact and incidental ingestion. This study investigated the potential human health risks associated with exposure to metals in soils at parks and playgrounds in Metro Vancouver, the third largest metropolitan area in Canada. One hundred and seven surface soil samples were collected from locations with high potential for human soil contact within 37 urban and peri-urban parks and playgrounds. The samples were analyzed for total metals, pH, total organic carbon and metal bioaccessibility. The relationships between metal concentrations and other physicochemical properties to point source emissions, surrounding land use and proximity to major traffic corridors were explored to ascertain the potential sources of the identified metal contaminants and factors which contributed to their introduction into the environment. Arsenic, copper, lead or zinc concentrations exceeding the Canadian Council of Ministers of the Environment soil quality guidelines for residential/parkland use were identified in soils from five of the parks investigated. Metals bioaccessibility values were variable; the mean values for select metals were As: 25%, Cd: 63%, Cr: 3.2%, Co: 12%, Cu: 33%, Ni: 11%, Pb: 51% and Zn: 24%. The results of the human health risk calculations incorporating bioaccessibility, the relationships between soil properties and bioaccessibility, and the plausible sources of the identified contaminants will be presented.

WE181

Assessing risks to soil function at a site impacted by DDT using the TRIAD approach

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For nearly 50 years a former division of the Swedish Forest Agency operated a plant nursery in Kårehogen located on the west coast of Sweden where different pesticides, including DDT, have been used. Today, investigations and remediation regarding on-site contamination is under the responsibility of the Geological Survey of Sweden. Previous studies have shown DDT concentrations in soil above the Swedish generic guideline value for sensitive land use (0,1 mg/kg), developed by the Swedish Environmental Protection Agency. Dimensioning for the value is the protection of the soil ecosystem. The objective of this study is to conduct a site-specific ecological risk assessment using the TRIAD approach (ISO 19204), where chemical, toxicity and ecological lines-of-evidence are evaluated to assess potential effects on soil ecosystem function at the Kårehogen site. Although the basic ideas of the TRIAD approach (e.g. the combination of information from chemistry, ecotoxicology, and ecology) have been accepted in Sweden, a national framework has not yet been developed, and its application in risk assessments of contaminated soils is rare. Thus, one additional objective is also to test the applicability of the TRIAD approach for soils contaminated with DDT. The same investigations and surveys were conducted at the impacted site and at a nearby reference site, which has similar soil physical and chemical conditions and vegetation cover, but with no evidence of use or impact by DDT or other pesticides. Concentrations of DDT and derivates were measured in 10 surface soil samples (0-30 cm deep) from each site. DDT in earthworm tissue was analyzed in 10 samples from the Kårehogen site. Effects on the invertebrate community were measured as abundance of earthworms, collembolas and nematodes, and species composition and biomass of earthworms at the 10 locations in each site. Effects on reproduction of earthworms (ISO method 11268-2) and collembola (ISO method 11267-2) were assessed using chronic toxicity tests on five surface soil samples from each site. The same soil samples were evaluated for potential impacts on the microbial activity using nitrogen and carbon tests (OECD tests 216 and 217). Results and the conclusion of the risk assessment will be presented. Additionally, the benefits and possible application of the TRIAD methodology in the approximately 40 other former plant nurseries currently administrated by the Geological Survey of Sweden will be discussed.

WE182

Soil vertical profiles and fate of Hg in different agricultural areas within a large historically contaminated site

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Among the national priority polluted sites, the SIN Brescia Caffaro is located in a mid size city, Brescia (200,000 inhabitants), in northern Italy. The Caffaro factory was among the largest former polychlorinated biphenyls (PCBs) producer in Europe and due to its chloro-alkali process, using mercury cells, released metallic mercury into the wastewater stream, which ended up in an irrigation canal. Such irrigation water was used in different crops for about 50 years, on about 100 Ha. In this project, Hg was measured in three different former agricultural areas (A, R, T, three different points per area), in vertical cores of up to 1 m depth. The resulting samples were representative of 0-10, 10-20, 20-30, 30-40, 40-60, 60-80, 80-100 cm. The three areas were selected based on their different use of soil: i) the points in area A belong to an area characterized by soil conditions typical of a mixture of grass and arable land, continuously irrigated, during winter, by contaminated waters (water meadow). Such situation could be responsible for oxygen content reduction in soil, by contributing to create prolonged anoxic conditions, favorable to Hg release; ii) the points in area R are representative of a former semi permanent grassland, before and after PCBs production, with a short break due to a conversion to arable land during PCBs production; iii) the points in area T are typical of an area which was characterized by intense plowing and continuous mixing of arable layer. These conditions were responsible for relatively large differences in concentration profiles among the areas. The results of concentration measurements with depth confirmed a general tendency of mercury to be confined to the upper 40-60 cm, while, in some cases, concentrations were higher at greater depths. A speciation analysis was also conducted. The results allow to reconstruct the soil concentration profiles during the historical contamination and to predict the vertical movement as well as the amount of mercury volatilized in time. Finally, such results can be also used to evaluate the potential losses of mercury to air and groundwater during the future phytoextraction-based remediation actions.

WE183

No-Till agriculture for reducing effects of groundwater salinity and contamination in corn cultures using experimental microcosm

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Agriculture is one of the most important and oldest economic activities in the district of Estarreja in Portugal. The irrigation of agriculture land is mainly based on the use of groundwater, exploiting the quaternary aquifer. Nevertheless, several studies showed the presence of nitrates, chlorates, organic and inorganic contaminants, and a high conductivity level in well waters. Observed contamination was mainly due to the various industrial activities in the area and in particular to the “Estarreja Chemical Complex.” The use of these waters to irrigate agriculture fields was expected to affect soil’s quality and increase their level of salinization, which interferes with plant nutrient absorption and alter the soil properties and functions. Thereby, it is expected that using the appropriate farming practices a sustainable production could be established. Several studies pointed out the importance of the no-tillage (NT) system farming for improving soil quality such as increasing soil porosity, reducing soil salinity stress (above 70%), and enhancing soil organic matter. In this context, the development of NT farming system can be beneficial. Therefore, with the present work it is intend to study the ability of NT farming strategies to minimize impacts on soil and corn (*Zea mays* L.) culturesirrigated with contaminated groundwater, comparing to the present farming practice (tillage farming). For this purpose, a plant-soil microcosm experiment was used. *Zea mays*was seeded and grown on agricultural soil after *Trifolium repens*sp (the white clover) decay, which was used as green manure, and left in soil surface (no till) or incorporated into soil (till). The evaluation of both practices was based on measuring physico-chemical changes in soils and some ecological indicators such biochemical and physiological parameters in treated agricultural soil and cultivated plants, respectively. The gathered results indicate that groundwater irrigation significantly increases soil’s salinization and contamination in both agriculture systems and resulted in inhibition of plants growth. However no-till system seems to be not sufficient to minimize damage in soils and cultivated plants. The results also reinforce the urgent need for studies to mitigate the negative impacts of irrigation practice in Estarreja.

WE184
Salinization effects on soil ecosystems: a SSD approach
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Salt-affected soil areas are increasing worldwide with negative consequences for soil fertility and biodiversity. Soil fauna and flora have been proved to be affected by saline conditions. However, no safety values of salinization for soil ecosystems have been derived. The aims of this study were: 1) to assess the effects of soil salinization on the reproduction and survival of soil invertebrates, and on the germination and biomass of agricultural plants, as well as 2) to derive safety values of salinization for soil ecosystems. To fulfill the first goal, the survival and reproduction of 8 soil invertebrate species (4 annelids and 4 arthropods), and the germination and growth of 6 agricultural plant species (3 monocotyledonous and 3 dicotyledonous) were evaluated through standard laboratory assays. The EC_{50s}were calculated for each species using the mean of the soil conductivity measured at the beginning and the end of each test (in a ratio of 1:5 soil:water fresh weight). Results showed that reproduction of soil invertebrate species was affected at lower salinity levels than their survival, with EC_{50s}ranging from ca. 50 μS/cm for the most affected earthworms to 900 μS/cm for the least affected mites. Regarding plant species, the parameter affected at lower salinity levels was the germination at the beginning of the test (IG), while no effect of salinization was found for root biomass. The sensitivities of both monocotyledonous and dicotyledonous seemed to be similar in terms of IG, with EC_{50s}ranging from ca. 600 to 1300 μS/cm. To fulfill goal (2), Species Sensitivity Distributions (SSD) were performed using the EC_{50s}obtained for soil invertebrate reproduction or the EC_{50s}obtained for IG of plants. Hazard conductivity values (HC) affecting 10 and 50% of the invertebrate species tested were ca. 180 and 820 μS/cm, respectively; while for the plant species tested, the HC10 and 50 were ca. 580 and 810 μS/cm, respectively. All values are below conductivity values measured in natural saline soils. For example, conductivity values measured using the same method in a South African soil is ca. 1650 μS/cm. At that salinity, the reproduction/IG of all tested invertebrate and plant species would decrease by at least 50%. The derivation of safety values for soil salinization towards soil ecosystems is of great need, but the sensitivity of communities in areas already exposed to saline stress should be also incorporated as their sensitivity might be different from non-exposed ones.

WE185
ECOLOGICAL SAFETY AND EFFECTIVENESS OF ANIONIC AND NON-IONIC SYNTHETIC SURFACTANTS FOR OIL-CONTAMINATED SOILS AND SLUDGE TREATMENT
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A reagent method for oil-contaminated soils and oil sludge treatment has several advantages and disadvantages. The benefits include the economy of clean water for soil washing, increasing the efficiency of dispersion separation, the possibility of extracting oil as a valuable resource, preparing the soil for subsequent phyto- and bioremediation. However, the reagent method is characterized by the secondary pollution with toxic substances, changes in the morphological characteristics of soils. Thus, the study of the chemical reagents toxicological properties is one of the important steps determining the surfactant applicability for the intended purposes. Synthetic surfactants used as chemical reagents for treatment (washing) of oil-contaminated soils and oil sludge must have met the environmental safety requirements. The research objective was determination of the biotoxicity level and effectiveness of an anionic and non-ionic surfactants. Laboratorial test was conducted for variety of surfactants; their biotoxicity was elucidated for the freshwater infusorians *Paramecium caudatum* Ehrenberg. The tests results would make it possible to create surfactant applicability matrices for various purposes. In this matrix graphically depicts the risks of selecting surfactants for oil-contaminated natural soils treatment. The matrix shows that highly effective, but toxic surfactants can't be considered applicable for the natural soils reclamation. However, these chemical reagents still can be used for oil sludge treatment, since after cleaning, their return to the environment is not provided. From the above, it follows that the choice of a chemical reagent for oil-contaminated soils or oil sludge treatment should be based on various requirements for environmental safety, toxicity of the surfactants under consideration.

WE186
A low cost, real-time measurements of CO2 uptake/emission as an early indicator of exposure to xenobiotics in plants and animals
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Respiratory rates of metabolically active organisms are objectively quantifiable, reflecting the magnitude of living processes essential for the survival of cells and organisms, in terms of its chemical products - particularly CO_2. Changes in environmental conditions as well as exposure to various xenobiotics result in alternations of metabolic activity that are reflected in CO_2 production/uptake dynamics. In this study we wanted to explore the usability of CO_2 uptake/emission measurement using low-cost sensors in continuous monitoring as an early indicator of exposure to xenobiotics in experimental organisms (plants and earthworms) and to compare it to different biochemical biomarkers. For the measurement of CO_2 an Arduino platform-based respiratory activity measuring system (ResTox) comprising sensors for measuring parameters relevant for the analysis of biological activity was developed. For plant experiments a *Lemna minor* was exposed to several metals: $HgCl_2$, $CuCl_2 \times 2H_2O$, $CdCl_2$ and $CoCl_2 \times 6H_2O$ and herbicides: diquat, clopyralid, nicosulfuron and tembotrione, separately. And for animal experiments three earthworm species (*Eisenia fetida*, *Dendrobaena veneta* and *Lumbricus terrestris*) were exposed to chlorpyrifos and copper oxide in nano form in both filter paper and artificial soil set-up. The results of CO_2 measurements in *L. minor* demonstrated that tested metals (Co, Cu, Hg, and Cd), as well as herbicides (nicosulfuron, diquat, and tembotrione), stimulated the CO_2 exchange rates at low doses, while at high doses CO_2 exchange rates were inhibited. CO_2 production data obtained in experiments involving earthworms exposed to chlorpyrifos and nCuO showed a dose-response type relationship between the concentration of toxicant and the amount of CO_2 produced, while the changes in measured biochemical biomarkers corresponded to those changes. With these experiments a continuous measurement of CO_2 exchange rates could be implemented as a promising tool for the initial screening of potential toxicity to obtain valuable information needed for further examination of toxicity mechanisms and risk assessment in both plant and animal organisms.

WE187
Statistical framework for regulatory risk assessment of field studies for soil organisms
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A regulatory risk assessment is performed for all Plant Protection Products (PPP). For the ecotoxicological risk assessment, a large range of organisms testing according to current guidance and assessment according to regulation 1107/2009 are required usually following a Tiered Approach. Regarding the soil organism, risk assessment covers worst-case situations (*i.e* conservative estimates and toxicity laboratory studies) to more realistic assessment (*i.e.* Field studies). A recent EFSA scientific opinion on "the state of the science on risk assessment of plant protection products for in-soil organisms" proposed a new framework for risk assessment of soil organisms and an revised protection goals defined for soil organism groups (*i.e.*, enchytraeids, arthropods etc…). Still, no statistical tool was made available to analyze field studies proposed for higher tier. The aim of the present work is to propose a statistical framework of existing tools already used for higher tier studies risk assessment at scale of population and communities. The statistical framework include community analysis that allow including the overall soil community decribed on the field. This last is based on multivariate analysis or generalized linear models. The population density and/or biomass are compared date-by-date using univariate test. The percent Minimum Detectable Difference (%MDD, Brock et al., 2015) should be also include to ensure the reliability of core data for the statistical analysis of comparison between treated and control populations. The ISO guidance for earthworm field studies (2014) provide the experimental setting recommendation for selecting the field, plots size and margin between them, as well as sampling and extraction of earthworm. ISO guidance on "Soil quality: effects of pollutants on earthworms" also provide some validity criteria especially regarding the sampled density of earthworm (number of individual /m²). Therefore, it is also noticed here that the %MDD may also be used to adapt the sampling strategy during the preliminary investigate of the chosen site to obtain a relevant number of earthworm (*e.g.* may be mitigate by increasing the number of plot or their size). This work could help to fill the current lack of harmonized statistical framework for field studies of soil organisms in the context of regulation of PPP.

WE188
SETAC Soils Interest Group
C. Lima, Vrije Universiteit Amsterdam / Animal Ecology
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Novel Developments in Testing Chemicals for Endocrine Disrupting Properties (P)

WE189
Plant protection products, endocrine disruptors and amphibians - where to start?
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Amphibians are gaining an increasing prominent role in the field of ecotoxicology. Especially, the lacking data and uncertainty regarding potential sublethal effects, as well as different modes of action of pesticides and endocrine disruptors in amphibians when compared to fish has been of increasing concern in the last few years. Recently, a scientific opinion regarding the need to evaluate the risks of pesticide applications to amphibians and reptiles was published by EFSA. Here, it became very clear that ecotoxicological data regarding these taxa is greatly lacking, and methodologies need to be adapted in order to complete a reliable risk assessment scheme. Similarly, regulations EC 1107/2009 and EC 528/2012 were recently amended in order to incorporate the assessment of endocrine disruptors with regard to aquatic organisms. It is generally accepted that in the majority of cases, acute toxicity of amphibians is covered by aquatic testing on fish. However, there are still many concerns whether this may also apply to sublethal effects such as development, gonadal development and metamorphosis. In light of the differing life-history and peculiar developmental biology of amphibians, this is of special importance. In an attempt to discern between the potential different sensitivities and effects of endocrine disruptors (e.g. pesticides) on these two different taxa, we conducted two tests using the same concentrations of an endocrine disrupting pesticide using *Oryzias latipes* and *Xenopus laevis* as test organisms.

WE190

Implication of Control Variability in Point Estimated-Based Assessment of Thyroid Disruption in the Amphibian Metamorphosis Assay

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In accordance with OECD Test No. 231 and USEPA Test Guidelines OPPTS 890.1100, the amphibian metamorphosis assay (AMA) represents a level 3 test within the OECD conceptual Framework for Endocrine Disrupting (EC) chemicals. The test exposes Nieuwkoop and Faber (NF) (5) stage 51 *Xenopus laevis* larvae to different concentrations of the test substance for 21-days and the following endpoints are measured: mortality, hind limb length (HLL), body length (snout to vent [SVL]), body weight, developmental stage, asynchronous development, and thyroid histopathology. Of these endpoints, SVL and body weight are considered measures of growth, whereas developmental stage, asynchronous development, HLL, and thyroid histopathology are considered in the assessment of thyroid axis disruption. Presently, guidance for the test provides a design for the establishment of concentration-based endpoints, but not ECx (i.e. EC10) values. Despite the ongoing regulatory discussion regarding the use of derived effect concentrations (ECx) rather than no effect (NOEC) values from chronic environmental studies, several requests have already been made by European member states to use ECx threshold in lieu of NOEC values. Specifically, the derivation of an EC10 value was requested by regulatory authorities for AMA studies. However, the biological variation of the test system was not considered resulting in uncertainty regarding the usefulness of this endpoint. The European Water Framework (WFD) Directive guidance document includes both EC10 and NOEC derived options, with no specific preference. However, additional investigation indicated that control variability in the AMA was greater than 10%, and thus the derivation of an EC10 value was of no biological relevance and therefore should not be used for regulatory purposes. To further evaluate the implication of control variability on the derivation of point estimates in AMA studies, control variability was assessed in 10 independently conducted AMA studies. Results of these analyses and the implications on the interpretation of AMA results will be presented.

WE191

Screening thyroid hormone disruption mechanisms of several chemicals using hepatic cell lines of human, rat, and zebrafish origin along with rat pituitary cell line

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Many chemicals that are frequently detected in humans can disrupt thyroid hormone balances. Since thyroid hormones play key roles in energy metabolism, growth, development, and reproduction, such disruption could lead to various health consequences. However, there are significant gaps in knowledge on mechanisms of the disruption, especially on metabolism in the liver. The present study was designed to understand the effects of several model chemicals on thyroid hormone metabolism using liver cell lines of different origin, i.e., human, rat, and zebrafish. For this purpose, human liver cell (Hep G2), rat liver cell (McA-RH7777), and zebrafish liever cell (ZFL) were chosen. In addition, a rat pituitary cell (GH3) was also used to understand the effect of these chemicals on central regulation of thyroid hormone balance. As test chemicals, five known thyroid disrupting chemicals (TDCs) based on either epidemiological or experimental studies, i.e., *p,p'*-DDE, PFOS, DEHP, BPA, and BP-3, were chosen. In addition, two more chemicals that are used in great quantity as sunscreen and plasticizer, i.e., EHMC, and DINCH, were also added. Following exposure to various concentrations of selected chemicals for 24 hours (Hep G2 and McA-RH7777) or 48 hours (ZFL and GH3), transcriptional changes of key genes were quantified using RT-qPCR. Exposure to PFOS and BPA significantly down-regulated *TBG* gene and up-regulated *PPARA* and *UGT1A1* gene in Hep G2 cells ($p < 0.05$). Regulatory changes of *DIO1* gene were observed but the direction varied by chemical substances. In McA-RH7777 cells, exposure to EHMC significantly down-reglated *Ugt1a1*, *Dio1*, *Ttr*, *Sult1b1* and *Ppara* genes ($p < 0.05$). However, the rest of chemicals did not show significant regulatory changes. In ZFL cells, exposure to BPA caused significant up-regulation of *ugt1ab* gene ($p < 0.05$). In addition, marked increase pattern of *ugt1ab* gene was shown following exposure to *p,p'*-DDE, DEHP, or BP-3 (>1.5 fold change). In GH3 cells, exposure to *p,p'*-DDE caused significant down-regulation of *Tshb* gene, while exposure to EHMC up-regulated *Tshb* and *Trhr* genes. The results of this study show possible species difference in hepatic metabolism of thyroid hormones or thyroid disrupting chemicals. Futher experimental studies are warranted to validate our observsations and to describe consequences in thyroid hormone regulation in vivo.

WE192

Thyroid disruption screening method using zebrafish vertebrate model

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Endocrine Disrupting compounds are being increasingly detected in the environment and may have a profound impact on the development and physiology of vertebrate organisms. Thyroid Disrupting (TD) compounds specifically alter the function of thyroid gland through the interference with the synthesis, transport and/or binding of the thyroid hormones. Several environmental contaminants such as polybrominated diphenyl ethers or halogenated organophosphates, used as plasticizers and flame retardants, are suspected to produce a TD effect. Given so, chemical manufacturing entities could benefit from cost-effective methodologies for the screening of TD substances in order to deselect candidates during the early phase of the development. The zebrafish vertebrate model is broadly used in both human and aquatic toxicity assessment due to their low cost, small size, rapid development, and homology with mammals. Besides, the existence of numerous transgenic strains enables to perform fluorescence-based screening assays with medium throughput. In this work, a screening method for TD substances assessment was developed using the Tg(tg:mcherry) transgenic zebrafish line. The fluorescence of the reporter gene allows monitoring *in vivo* the upregulation of the thyroglobulin gene expression as a compensatory reaction to thyroid gland disruption. In this assay, the exposure concentrations of test substances were selected according to the results of a preliminary acute toxicity assay to avoid any interference by non-specific toxicity. Afterwards, transgenic embryos were exposed to the test substances from 48 to 120 hpf (hours post-fertilization) and subsequently imaged by fluorescence microscopy. A dose-dependent increase of the fluorescence was observed, and the intensity values were fitted to a concentration-response regression model to calculate TD predictors, such as the Benchmark Concentration (BMC), Thyroid Disrupting Index (TDI), and Relative TD Potency (RTP). Finally, an rt-qPCR gene expression assay was developed over known markers of thyroid pathway (tshβ, tg, and tpo) to characterize the mechanism of action involved in the endocrine disrupting effect. The screening method presented in this work has been developed and validated using a set of 19 environmentally relevant TD substances. This screening methodology showed to be a sensitive and cost-effective assay to evaluate the potential thyroid disruptor activity of chemicals.

WE193

Toward an AOP-based tiered testing strategy for thyroid hormone disruption in fish

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Assessment of thyroid hormone disrupting chemicals (THDCs) is considered a major gap in current approaches for the testing of endocrine disrupting chemicals. The thyroid hormone system is involved in several important physiological and developmental processes, and many chemicals found in our environment may interfere with the thyroid system. The need to advance screening and testing strategies for THDCs includes the development of *in silico* and *in vitro* methods that anchor chemicals acting through a thyroid mechanism to adverse responses.The Adverse Outcome Pathway (AOP) framework may therefore be particularly helpful for the identification of relevant assays and endpoints. Here, we demonstrate the use of *in chemico* assays targeting specific key events of an established AOP network to predict higher biological endpoints in fish early-life stages. Specifically, an AOP network linking thyroid hormone disruption to impaired swim bladder inflation was used to select assays measuring thyroperoxidase and deiodinase inhibition, key enzymes in the thyroid hormone metabolism. A set of 51 compounds was screened using these assays, and data were used to predict acute and chronic effects on swim bladder inflation. Predictions were validated using FET and FELS *in vivo* experiments in zebrafish and fathead minnow, and *in chemico* to *in vivo* extrapolation threshold values were established. A tiered testing strategy for the identification of THDCs was proposed based on these data. Our thyroid hormone disruption AOP network is part of the OECD AOP development programme workplan (project 1.35), and the associated assays align with the thyroid-related assays that are listed in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (revised 2018) as assays forwhich no formal guidance has been written at present. In addition, as part of their endocrine disruptor screening program, the US EPA included this work while assembling a conceptual thyroid hormone disruption AOP network spanning different taxonomic groups (fish, amphibians, mammals) to assist high throughput assay development. The ongoing JRC EURL ECVAM validation effort of *in vitro* assays for THDCscreening is making use of this project's data to ensure synergies and overlap. Finally, our AOP network will be used as background for the H2020 project ERGO, "Breaking down the wall between human health and environmental testing of endocrine disrupters".

WE194

Thyroid disruptors interfere with eye development and function in zebrafish

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Vertebrate eye development is partly regulated by thyroid hormones (THs). We investigated the effects of thyroid-disrupting chemicals (TDCs) on eye development of zebrafish at morphological and transcriptional level. To this end, zebrafish embryos were exposed to either propylthiouracil (PTU), a TH synthesis inhibitor, or tetrabromobisphenol A (TBBPA), which interacts with TH receptors, until 5 days post fertilization (dpf). The TDC exposure induced reduction of eye size and pigmentation together with altered cellular retina structure. We expected the TDC exposure to cause transcriptional changes of visual-system-related genes, which find their phenotypic anchoring in the observed eye malformations. Moreover, we investigated if the different TDC modes of action would affect similar molecular pathways in the eyes. Full genome microarray analyses of RNA isolated from eye tissue revealed that the number of affected transcripts was substantially higher in PTU- than in TBBPA-treated larvae. However, multiple essential components of phototransduction (e.g. phosphodiesterase, opsins) were responsive to both TDC exposures. Yet, the response pattern for the GO-class "sensory perception" differed between treatments, with over 90% down-regulation in PTU-exposed fish, compared to over 80% up-regulation in TBBPA-exposed fish. Additionally, the reversibility of effects after recovery in clean water for 3 days was investigated. Transcriptional patterns were still altered, and partly overlapped between 5 and 8 dpf, showing that no full recovery occurred. However, pathways involved in repair mechanisms were significantly upregulated, which indicates activation of regeneration processes. These results confirm the growing evidence that fish eye development is sensitive to TDC treatment and might represent a promising endpoint for the assessment of thyroid-related effects in fish.

WE195

Interference of hepatotoxicity with endocrine activity in fish

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Vitellogenin (VTG), a well-established biomarker for the diagnosis of endocrine activity in fish, is used in multiple OECD test guidelines to identify (anti)estrogenic activities of test compounds. However, the synthesis of VTG may not only be modified by typical endocrine-related pathways, but also through non-endocrine-mediated processes. In particular, hepatotoxicity, i.e. toxicant-induced impairment of liver structure and function, might influence VTG as a biomarker, since it is synthesized in hepatocytes. An intimate understanding of the interplay between primary endocrine-related and non-endocrine-related pathways influencing VTG production is crucial for the avoidance of false diagnoses in hazard assessment, since endocrine disruption is used as a cut off criterion for biocide and plant protection product active substances in Europe. To investigate whether hepatotoxicity may interfere with VTG synthesis in the liver of exposed fish, effects by three well-known hepatotoxicants, acetaminophen (APAP), isoniazid (INZ) and acetylsalicylic acid (ASA), were tested in adult zebrafish (*Danio rerio*) in a 21-day flow-through exposure test according to OECD test guideline 230. Various hepatotoxicity- and endocrine system-related endpoints were recorded: mRNA expression of selected endocrine-related and hepatotoxicity-related marker genes in the liver; protein levels of VTG in head/tail homogenates; liver histopathology. All three test compounds induced mild, but significant single cell necrosis of hepatocytes and transcriptional changes of the hepatotoxicity-related marker genes, which confirmed the hepatotoxic effects of the chosen compounds, even though effects were not pronounced. Only minor changes in VTG levels were observed, which were neither compound- nor gender-specific, nor did the substances evoke a uniform VTG response. Likewise, transcriptional responses of endocrine/estrogen-related genes in the liver were sex- and compound-dependent and indicating that the hepatotoxicants investigated did not have major (indirect) impacts on endocrine activity. The results show that a clear correlation between hepatotoxic effects and VTG synthesis in the liver could not be demonstrated in the chosen exposure scenario. This suggests that − under the exposure conditions of OECD TG 229 and 230 − it is unlikely that chemicals cause hepatotoxic effects interfering through this mode of action with the hepatic capacity for VTG synthesis.

WE196

The Extended one generation reproduction test with zebrafish, first results from a validation study with tamoxifen-citrate

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After acceptance and enforcement of the criteria for identification of endocrine acting substances (EAS), there is increasing need for valuable testing strategies and methods. In this context, fish populations are considered as main targets of endocrine acting compounds in the aquatic environment. In 2015, OECD adopted a protocol for an Extended one generation reproduction test (EOGRT) with Medaka (*Oryzias latipes*). This test protocol includes the exposure of a parental generation (adult fish), a full Filial 1 (F_1) generation (from egg to adult) and a Filial 2 (F_2) generation (until hatch of embryos). As this protocol was designed and validated for a single test species, there was an initiative from Germany to develop a similar test approach considering zebrafish (*Danio rerio*) as a further species. The development of the Zebrafish EOGRT protocol was placed on the OECD workplan in 2016. The protocol considers the timeline as developed for the Medaka EOGRT but adds some method adjustments like true replication of test tanks, absence of tissue samplings during the test and the focus on more species-appropriate settings. Due to the lack of a gene locus defining the genetic sex, a genetic sex determination is excluded from the Zebrafish procedure. First results from a validation study with tamoxifen citrate, a known estrogen receptor antagonist, are available. The data obtained were in line with literature data for zebrafish and other fish species. Comparable sensitivity was observed for e.g. reproduction parameters in terms of fecundity and fertility. A shift in sex ratio towards an increased number of males was observed and defined as the most sensitive endpoint with population relevance. Biomarker measurements revealed a decrease of vitellogenin concentrations in female blood plasma samples. Decreased survival rates for juvenile fish of F_1 and F_2 generation showed corresponding effects levels. It can be postulated, that these effects were the result of an impacted egg qualily. These results confirm the applicability of the test protocol. The data obtained is valuable to identify sensitive endpoints and gain mechanistical information used for ED assessement.

WE197

The impact of synthetic progestins on fish populations: Results from a Zebrafish EOGRT with Dienogest

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The intake of endocrine acting substances (EAS) can represent a relevant threat scenario for the aquatic environment. Due to a very direct route of exposure, fish populations are recently in the main focus of environmental science and research. The release of human pharmaceuticals and veterinary drugs is steadily increasing. Moreover, the progress in pharma science aiming to design new active pharmaceutical ingredients (API) results in the development of sufficiently stable, specific and potent molecules. There are many examples for substances acting already at very low concentration levels and finally causing adverse effects in the aquatic environment, expecially on fish populations. The regulation of pharmaceuticals increasingly considers the impact on the environment. A current project of German Environment Agency (UBA) aims to develop a tailored risk assessment strategy for the group of new synthetic progestins and glucocorticoids. Effects on aquatic vertebrates, invertebrates and sediment dwellers should build a data base for developing this adapted assessment strategy. Here we present results from a Zebrafish extended one generation reproduction test (ZEOGRT). The synthetic progestin dienogest was chosen as test substance. The study followed a new test protocol for a two generation test with zebrafish (*Danio rerio*), including the continuous exposure of a parental generation (adult fish), a full filial 1 (F_1) generation and finally a filial 2 (F_2) generation (hatch only). The results showed no impact of dienogest exposure to reproduction capability of the parental generation. However, a clear reduction of F_1 post hatch survival was found. Further endpoints to be assessed will be life stage specific growth and survival rates, reproduction in terms of egg number and fertility for F_1, and sex ratio. The available results confirmed the applicability of the chosen study protocol and already show an increased sensitivity of fish compared to other inhabitants of the aquatic environment, also exposed to progestins.

WE198

A lifetime exposure to bisphenol S up to 10 mg/L does not adversely affect growth, reproduction or sex ratio of zebrafish

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Bisphenol S (BPS) is used as an intermediate (monomer) in the production of high performance plastics and other polymers and discussed as an alternative for bisphenol A in thermal paper. Several published studies have investigated the effects of long term exposure to BPS on zebrafish and reported endocrine activity at μg/L concentrations. However, there is no consistency among the published studies on the chronic exposure level which results in systemic or reproductive toxicity to zebrafish. In response to an ECHA decision on a substance evaluation for BPS, we conducted a range finding study in zebrafish with the objective to identify a 32-d growth LOEC to use as the high concentration for a definitive extended one generation reproduction test (EOGRT). The study consisted of a negative control and the following concentrations of BPS in groups of 2 replicate aquaria (20 eggs/rep): 0.0032, 0.01, 0.032, 0.1, 0.32, 1.0, 3.2, 10 mg/L. A broad concentration range was investigated in response to the disparity among the published results and exposure concentrations were verified. No adverse effect on growth in live fish was identified up to 10 mg/L after a 32-day exposure (NOEC ≥10 mg/L), therefore the range finder was extended for up to 148 days, covering one full zebrafish generation, in an attempt to define a LOEC on an apical endpoint. Observations of statistically reduced growth in our study were not consistent over time nor was there any concentration response relationship or discernable trend in the data and thus could not be linked to BPS exposure. Over the entire test there were no statistically significant exposure related effects or trends on survival, reproduction (as fertility and fecundity) or sex ratio. The overall NOEC was ≥10 mg/L for apical endpoints under the conditions of this study. The results of this 148 day study of zebrafish over one full generation do not confirm the reported adverse effects of BPS on reproduction and growth in previously published studies with considerably shorter exposures. Since only 2 replicates were included in the study design, definitive conclusions on subtle effects can not be drawn; however given that the exposure extended up to the chronic fish limit concentration, 10 mg/L, it is unlikely that severe chronic toxic effects were overlooked in this study. A definitive assessment of the potential chronic toxicity and adverse endocrine effects of BPS will be evaluated in a zebrafish-EOGRT.

WE199

Exposure to Norgestrel (NGT) and norethindrone (NET) in Gambusia affinis: Effects and Mechanisms

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Natural and synthetic progestins in receiving streams can disrupt the normal endocrine systems of fish. Norethindrone (NET) and norgestrel (NGT) are synthetic progestins widely used in oral contraceptives and frequently detected in wastewater effluents. The adult female western mosquitofish (*Gambusia affinis*) were exposed to NET or NGT at environmentally relevant concentrations for 42 d. NGT caused a rapid masculinization of females, an increased frequency of atretic follicular cells in ovaries and impaired mating behaviors exhibited by males toward the NGT-exposed females. Exposure to NET led to rapid masculinization, altered histology of the livers and ovaries. In addition, exposure to NET did not affect the expression of the androgenic and estrogenic receptor genes and *Cyp19a* except for a significant up-regulation of *Era*. However, the expression of *Vtg A*, *Vtg B*, and *Vtg C* were markedly inhibited in the females. Relative to the control females, exposure to NET stimulated the expression of *Hsd17b3* in the livers. The results demonstrate that NGT and NET can cause rapid masculinization of female *G. affinis*, changes in histology of the livers and ovaries, and alterations in genes related to endocrine systems. The results imply that *G. affinis* populations might be threatened in NET- and/or NGT-contaminated environments.

WE200

Comparison of endocrine effects in different life-stages of zebrafish exposed to anti-estrogenic/androgenic substances in varying life-cycle exposure scenarios

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During the authorization process of plant protection products a series of toxicity testing has to be performed to ensure an acceptable risk of use for humans or the environment. In these assessments fish populations represent an important spot in ecosystems and are tested for short- and long-term constraints accordingly. Of high interest in the procedure of testing are effects upon the endocrine system as they may alter e.g. the reproductive fitness of adult fish or their progeny. Recently, scientific criteria for the identification of endocrine disruptors (EDs) have been set up by the EC. But despite a long history of research there is still a debate about mechanisms of action of EDs affecting the legislative regulation. These uncertainties arise mainly from the occurrence of non-monotone concentration-response relationships and the applicability of the concept of effect thresholds. To answer these questions we exposed zebrafish (*D. rerio*) during different phases of their life-cycle to an anti-estrogenic substance, tamoxifen citrate, in both a pulsed and a permanent exposure test setup. The pulsed exposure experiments were performed using a spiked water-sediment system, in which three different developmental stages were exposed in parallel. A concentration-dependent mortality occurred in a first experiment (125 μg/L – 1000 μg/L). Nonetheless, in concentrations not affected by mortality and thus no subject to systemic toxicity a shift of the sex ratio towards males was observed. As no NOEC could be established for this sensitive endpoint, the experiment was replicated applying a lower concentration range (12.5 μg/L – 125 μg/L). Similar effects were observed when fish were constantly exposed to the test substance following the protocol of the Zebrafish Extended One Generation Reproduction Test (ZEOGRT), currently undergoing the OECD guideline validation process. Adult fish were exposed to concentrations ranging from 0.2 μg/L to 20 μg/L during their reproductive phase, followed by life-long exposure of their offspring (F_1) until hatching of the F_2-generation. The shift to male animals was conclusively observed in fish of the F_1-generation. Further results from this project will deliver additional information with respect to type and time of onset of effects. The data will be complemented with available data for other EDs (anti-estrogenic/androgenic) using similar testing approaches but covering differences in bioavailability prior to test item degradation.

WE201

Androgen antagonists: are tests conducted on adult fish able to capture activity due to this modality?

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Compounds acting as androgen antagonists (AACs) may interfere with the endocrine system in different ways such as by competitively binding to the androgen receptor (AR), blocking its activation, and/or subsequently interfering with the expression of genes regulated by the AR. In contrast to estrogens, androgen antagonists have not been well characterised in non-target organisms. In fish, AACs can lead to impaired gonads and sex secondary characteristics development, feminization of males, impaired reproductive behaviours, and reproductive failure in females. Furthermore, adult males can show altered reproductive behaviour such as nest production and courtship behaviours. All those effects can ultimately reduce fertility and fecundity being, therefore, very relevant for the maintenance of the population. In 2018, EFSA and ECHA published a guidance for the implementation of the Regulation 2018/605 laying down the criteria for the hazard identification of endocrine disrupters. Several scenarios are identified based on the available data package and, for each scenario, the testing strategy or recommendations on how to proceed in the assessment are given. In case further data with non-target organisms are needed to elucidate the endocrine activity due to estrogenic, androgenic and steroidogenic modality, level 3 (*in vivo* mechanistic) tests with fish are recommended (OECD TG 229 and OECD TG 230). Those tests are performed on adult fish which are exposed for 21 days to the compound under assessment. Parameters like VTG, gonad histopathology and fecundity/fertility are measured. As reported in literature effects of known compounds acting as anti-androgens are not consistent. Anti-androgens can impair sexual differentiation when early life stages of fish are exposed. However, in case adults are exposed there could be cases where effects due to anti-androgenic activity are not detected for example because fish were exposed after the sexual differentiation occurred. To this aim, additionally available OECD test guidelines (e.g. OECD 234) and guidance (OECD 148) will be considered and their strength and weaknesses will be highlighted as possible alternative solutions to better cover compounds acting as androgen antagonists.

WE202

A novel transgenic zebrafish line (cyp19a1a-GFP): refinement of an OECD test guideline for evaluating the effects of prochloraz on aromatase gene expression and reproduction.

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Concern about the effects of endocrine disrupting chemicals (EDCs) on reproductive health has stimulated the development of mechanism-based models and assays. In this regard, transgenic fish are powerful biological models that can provide mechanistic information regarding the endocrine activity of test chemicals. The objective of this study is to assess the feasibility of using a new biological model in an OECD fish screening assay to provide additional mechanistic information, as compared to wild-type models. In the present study, we used a transgenic zebrafish line *cyp19a1a*-GFP expressing GFP under the control of the "ovarian" aromatase promoter. Based on the OECD Fish Short Term Reproduction Assay (TG229), the effect of a prochloraz (PCZ; 3, 30 and 300μg/L), was assessed on "classical" endpoints. Additionally, fluorescence of ovarian GFP was monitored *in vivo* at 4 times from T0, to determine the baseline level of fluorescence, to T21 days of exposure to determine the kinetic of perturbation. After 21 days of exposure, a significant decrease in the number of eggs laid per female per day was observed at 300 μg/L. PCZ led to a concentration-dependent inhibition of circulating vitellogenin concentrations in females most probably reflecting the decreasing E2 synthesis due to inhibition of ovarian aromatase activity. GFP intensities were similar over treatment groups at 7 and 14 days of exposure but significantly increased after 21 days of exposure at 30 and 300 μg/L. A similar profile was observed on the endogenous *cyp19a1a* gene expression analyzed by qPCR. The overexpression of the aromatase gene likely reflects a compensatory response to the inhibitory action of PCZ on aromatase enzymatic activities consistent with the literature on PCZ. Thus, the *cyp19a1a*-GFP transgenic zebrafish line allows *in vivo* non-invasive monitoring over time of the GFP fluorescence of the ovary, providing information on aromatase expression and its perturbation. The protocol developed for GFP measures has no observable effects on reproduction and survival of fish. Moreover, it allows evaluation of EDCs effects over time and concentrations. Taken together, our findings highlight that the transgenic *cyp19a1a*-GFP model is a sensitive and relevant tool, which can bring complementary data without increasing the number of individuals or costs of the experiments.

WE203
Multiplex gene expression platform for screening endocrine disruptors using zebrafish
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Acute and long-term exposure of living organisms to environmental chemicals have been related to a wide range of physiological alterations and adverse health outcomes. Although mechanisms of action remain mostly to be elucidated, there is growing evidence that some of these effects may be due to changes at level of endocrine system. Zebrafish has been proven an excellent experimental model to screen endocrine disruptors, but current zebrafish-based assays to assess endocrine disrupting potencies (EDPs) have not been standardized yet, meaning that there is not consensus on endpoints to be measured. Moreover, experimental conditions may vary depending on the assays and the endocrine pathway evaluated. With the aim of providing a simple, robust and fast assay to assess EDPs for the three major endocrine axis, i.e. estrogens, androgens and thyroid, we propose the use of a panel of 8 gene expression biomarkers in zebrafish larvae. This includes brain aromatase (*cyp19a1b*) and vitellogenin 1 (*vtg1*) for estrogens, cytosolic sulfotransferase 2 family 2 (*sult2st3*) and cytochrome P450 2k22 (*cyp2k22*) for androgens, and thyroid peroxidase (*tpo*), transthyretin (*ttr*) thyroid receptor α (*trα*) and iodothyronine deiodinase 2 (*dio2*) for thyroid metabolism. All of them were selected according to their responses after exposure to the natural ligands 17β-estradiol, testosterone and 3,3′,5-triiodo-L-thyronine (T3), respectively, and subsequently validated using compounds reported as endocrine disruptors in previous studies. Cross-talk effects were also evaluated for all compounds.

WE204
EFFECTS OF 4-NONYLPHENOL AND 17β- ESTRADIOL ON SYNTHESIS OF VITELLOGENIN AND BALANCE OF STREROID THYROID HORMONES IN Sexually immature yeallowfin seabream, Accanthopagrus Latus
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In the study, the esterogenic effects of 4-nonyphenol (4-NP) and 17β-estradiol (E2) and on synthesis of vitellogenin (VTG), level of steroid and thyroid hormones and hepatosomatic index (HIS) in immature yellowfin seabream, Accanthopagrus latus, were assessed. To do this, a total number of 104 fish were abdominally injected by 10, 50,100, 150 and 200 μg/g/week and 2 μg/g/week of E2. The experiments were conducted over a period of two weeks. Induction of vitellogenin generation in plasma of treated fish was assayed indirectly by measuring of total plasma calcium and alkali-labile phosphate. The increased level of the indicators in treated fish's plasma showed the production of 4-NP in liver dose-dependently changed. Meanwhile, a significant dose-dependent increase was observed in HIS which indicated the activation of hepatic VTG production in 4-NP- and E2-injected fish. 4-NP treatment did not have any significant effect on plasma levels of testosterone. In addition, it was observed that 4-NP affect the level of thyroid hormones in fish. Plasma thyroxine levels rose in a dose-dependent manner after 7 and 14 days of the exposure. In contrast, a significant decrease in triiodothyronine levels was observed during the experiment period. Moreover, no significant change was detected for thyroid stimulating hormone levels in 4-NP-treated fish. These results demonstrated that 4-NP is so influential to induce the VTG. Based on the findings, it can be concluded that 4-NP can strongly disturb the balance of steroid and thyroid hormones with potential consequences for sexually immature male yellowfin seabream

WE205
Do the principals analogues of bisphenol A have endocrine activity? In vitro case study.
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Endocrine disruptors (EDs) can affect the different levels of epigenetic control and in some cases can act transgenerationally, if the exposure to EDs occurs during "critical windows of exposure", especially, the prenatal and the early life period. In this study, it was selected Bisphenol A (BPA) and two analogues of it, Bisphenol S (BPS) and Bisphenol F (BPF). These chemicals are wide spread distributed, and it is known that the prenatal or early exposure to BPA is related with two potential diseases, obesity and diabetes in childhood as well as reproductive, behavioural and neurodevelopment problems. Previous studies described an obesogenic activity of BPA. This compound can affect the expression of master regulatory genes related with adipogenesis such as the peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein (C/EBPα). Nowadays, the European Commission (EC) has imposed some regulations, such as the withdrawal of baby products containing BPA. However, the principal analogues of BPA (BPS and BPF) are still in them. The overall objective of this project is to compare the obesogenic activity of BPA and its analogues by *in vitro* assays with preadipocytic 3T3-L1 cell line. To proceed with that, MTT assay was performed in order to select the concentrations of each chemical, which did not show a cell viability decrease. Consequently, it was evaluated the adipogenic differentiation capacity of these chemicals by OIL RED O cell staining. Furthermore, the transcription of the master regulatory genes involved in 3T3-L1 differentiation were assessed by real-time PCR (to determine genetic expression) and western blot (to determine protein expression). The results showed a greater toxic effect of BPA at lower concentrations than its analogues. However, BPS followed by BPF showed a greater obesogenic capacity than BPA, which was reflected in an increase in C/EBPα and PPARγ compared with control. This same pattern could be observed in the stain with OIL RED O. As a conclusion of the study, these analogues could present even greater endocrine activity than BPA.

WE206
The fish sex ratio endpoint in the assessment of endocrine disruption
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In recent years, the scientific community and authorities have been discussing the endocrine disruptors topic and how to regulate it. Significant progress has been achieved in the EU, with the establishment of scientific criteria to identify substances with endocrine disrupting properties both in the Plant Protection Products Regulation (EC) 1107/2009 and the Biocidal Products Regulation (EU) 528/2012, and the publication of the EFSA and ECHA's Guidance Document on the identification of endocrine disruptors (EDs). According to this guidance document it is required to consider for EDs assessment, beside regulatory test results, all already existing data, including available literature data. Sex ratio is an endpoint included in several fish test guidelines, addressing both adversity and endocrine activity, two essential elements for EDs identification for regulatory purpose. However, the interpretation of this endpoint is not always straightforward, due both to an incomplete understanding of the underlying rational of its potential variation and to the diversity of experimental design found in literature studies. The aim of this work was to appraise available sex ratio results in the scientific literature for regulatory use. More than 100 articles published in the last 20 years, reporting data on the sex ratio in zebrafish, Japanese medaka and fathead minnow, have been compiled and assessed. The different methods for quantification of sex ratio have been compared, and several characteristics of the sex ratio have been further investigated, such as the plasticity of the endpoint according to the lifestage and the biology of the species; the reversibility of the endpoint that might be observed in some circumstances; and the natural variability of sex ratio reported in control fish along with the factor influencing it such as the fish strain or culture condition, including temperature. The results will be discussed to better understand the extent to which the different factors can significantly influence the sex ratio endpoint, in relation to the species sensitivity and the test design. Finally, the sensitivity of the sex ratio endpoint will be compared to the sensitivity of other endpoints related to endocrine disruption identification, and the possible use of this sex ratio data for identification, classification, testing and risk assessment in the regulatory context will be discussed.

WE207

The new ECHA/EFSA Guidance for the identification of endocrine disruptors - initial experiences with the assessment for aquatic vertebrates

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The Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market states that an active substance shall not be approved if it is considered to have endocrine disrupting (ED) properties that may cause adverse effects on non-target organisms (i.e. a cut-off criterion). In April 2018, the European Commission has implemented scientific criteria for the hazard-based determination of endocrine-disrupting properties pursuant to Regulation 1107/2009. According to the ED criteria, a substance shall be considered as having ED properties in non-target organisms (other than humans) if it meets the following three criteria: a) it shows an adverse effect that is relevant for non-target organisms at population level, b) it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system, and c) the population-relevant adverse effect is a consequence of the endocrine mode of action. In June 2018, ECHA and EFSA published a joined Guidance Document intended to provide guidance to applicants and assessors of the competent regulatory authorities on the implementation of these criteria. The Guidance Document describes how to gather, evaluate and assess all relevant information, conduct a mode of action analysis, and apply a weight of evidence approach, to establish whether the ED criteria are fulfilled. This poster shares the first practical experiences with the new Guidance Document concerning the ED assessment focussing on aquatic vertebrates. It provides an applicant point of view on the assessment procedure, data sufficiency, and potential testing strategies.

WE208

Endocrine assessments for pesticides and biocides - a regulators perspective

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Following the adoption of the criteria for endocrine disruptors (ED) for pesticides (Commission Regulation (EU) 2018/605) and biocides (Commission Delegated Regulation (EU) 2017/2100) it has been necessary for Rapporteur Member States (RMS) to use information provided by applicants to perform an assessment of the ED properties of the active substance to submit to EFSA / ECHA for peer review. Whilst there are some common elements the different regimes have also presented slightly different situations, so our experience with pesticide active substances, biocide active substances and biocide products are described. The UK has adopted a stepwise approach when preparing the ED assessment in order to reach a robust conclusion as efficiently as possible without requesting additional vertebrate data unless it is essential. This approach and the reason for this approach will be explained in the presentation. The data package for pesticides is typically larger, particularly with respect to chronic data than for biocides, which has resulted in different ED assessments being submitted by applicants. For both regimes we will outline our experince to date with examples of what information we had available to reach the different conclusions. In both cases a human health data package is available which is evaluated initially to consider whether the substance fulfils the criteria that the substance is a ED for human health, which if positive can be used to then assess the population relevance of the effects seen to address the non-target organisms assessment. Our experiences to date have indicated that unless it can be demonstrated that a substance meets the scientific criteria described under the implementing regulation there is often insufficient information to reach a conclusion. As a result our conclusion has frequently been that the scientific criteria described under the implementing regulation are not met, but there is insufficient information available to conclude on the criteria which confidence. This triggers a need for further data / information generation after which time the assessment needs to be repeated.

WE209

EFSA new guidance on endocrine disruptors: comments, critical aspects and a case study

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The EFSA Endocrine Disruptors guidance has been developed to help assessors of the regulatory authorities on the definition of the scientific criteria for the determination of endocrine-disrupting properties in the context of Regulations (EU) No 16 528/2012 and (EC) No 1107/2009. The overall objective resulting from the present project was the compilation of a specific database for a pesticide active substance (named x) and its metabolites, which is comprising all available parameters that are considered relevant when investigating the ED properties. The database represented a practical tool to help assessors in assessing and analyze the evidence for endocrine disrupting properties. A useful-friendly client-side Form of the database was created. The Microsoft Access database was selected as suitable for this specific data collection due to the number of predicted records to be stored in and the ability of the toxicologist to work with this specific platform. To facilitate the data entry phase of the operators and to limit the data entry typos, most of the field were constrained with menu combo boxes and several queries were prepared to check the quality of the inserted data. The list of toxicity study type for mammalian toxicology and wild life toxicology comprises more than 10 different type of studies. Out of 55 studies that were scrutinized, 27 were performed on the active substance and 28 were performed on metabolites (4 metabolites) thus representing more than 50% of the total. In this particular case, the number of toxicity studies performed on metabolites was larger than normal; this is due to particular toxicity potential of three (non mammalian) metabolites that show higher chronic (specifically reproductive toxicity) toxicity than the parent. Studies on mammals (intact organism/cells) were 49 (23 on active substance and 26 on metabolites), while studies on wildlife (fish, amphibians) were 6 (4 on active substance and 2 on metabolites) Wilde life studies to investigate ED properties of active substances were not routinely performed in the past year. Please note that for substance X dossier year of study range from 1979 to 2012. Collection of data from substance x dossier represented a new exercise related to new approach for evaluating ED properties of active substances.

WE210

What to expect from the evaluation of endocrine disrupting properties: hazard versus risk-based approach - an update

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Should we assess the potential endocrine disruptive properties of plant protection and biocidal active substances based on hazard or risk? In other words, is the public health and the environment better off with an assessment of the hazardous properties of an active substance to conclude on its potential endocrine disruptive properties, or on management of the exposure? These questions are answered differently in the European Union (EU) and the United States (US). The current case study focuses on the distinct approaches taken in the EU (hazard-based) and US (risk-based). In the EU, very few substances have actually been 'banned' so far based on the conclusion of endocrine disruptive properties and most of the case-by-case ED assessments up until now have been inconclusive. With the publication of the 'Guidance for the identification of endocrine disruptors in the context of Regulation (EU) No 528/2012 and (EC) No 1107/2009' (ECHA and EFSA, 2018) this may change, as finally a framework for evaluation of ED-relevant testing data has become available. In the US, in contrast, an Endocrine Disruptive Screening Program (EDSP) is in place since several years. Tier 1 screenings of selected active substances have led to the identification of chemicals with and without potential to interact with the oestrogen, androgen or thyroid pathway. Of the 52 chemicals considered for Tier 1 screening, already 34 were concluded not to pose risks for endocrine disruption. Of the remaining 18 chemicals, all 18 show potential interaction with the thyroid pathway, 17 of them with the androgen pathway, and 14 also potentially interact with the oestrogen pathway. For these substances, further studies to be generated were identified. In the present case study, we investigate the lessons that can be drawn from the US screening program and possible implications for the ED assessments to be conducted in the EU under the different regulatory frameworks (REACH, BPR, PPPR).

WE211

Use of High Throughput Screening assays to develop local (Q)SAR models on a specific chemical category: A case study on aromatase activity of conazoles

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In the last years concerns were raised toward the potential hazard posed by endocrine disrupting chemicals (EDCs). Developing low tier testing methods, like *in vitro* and *in silico* ones, is important to meet the 3R principles giving an effective assesment of potential endocrine disruption. The aim of this work is to develop a Quantitative Structure Activity Relationship (QSAR) model giving a quantitative estimation of the aromatase inhibition potential of conazoles. Conazoles are commonly used antifungal agents and act as C-14 demethylase inhibitors, that shows a high homology with human aromatase. Data were collected from literature to verify the correlation between a selected high throughput assay and the most used *in vitro* tests used in regulatory framework for Plant protection Products (PPP). The results confirmed a good correlation among different tests. Case weighted Random Forest (CWRF) algorithm was applied for QSAR development. This approach uses a large heterogeneous training set but it gives more emphasis to a subset of selected observations during model's derivation (i.e., conazoles). The aim is to develop a model for a specific class of chemicals. Results demonstrated that the method is suitable to assess aromatase inhibition potential of conazoles outperforming classical and local QSAR models.**Acknowledgements:** This study was funded by EU-ToxRisk (Project reference: 681002).

WE212

Virtual Cell Simulations to Probe the Effects of Complex Mixtures from Low to Higher Concentrations

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People and ecological receptors are exposed to chemicals as mixtures, not as single chemicals. However, there still exists a great deal of uncertainty with respect to how chemicals interact at low and higher environmental concentrations. The goal of this project is to use the estrogen receptor and gene expression response as a cell signaling model to explore how chemical mixtures respond both in vitro (i.e., the absence of endogenous estrogens) and in the likely in vivo situation (where endogenous estrogens are also present). The model uses known equations from biochemistry and affinity/dissociation constants for chemicals, and estrogen receptor binding to DNA, and gene expression kinetic models to integrate knowledge. We used an agent-based system, where chemicals, the estrogen receptors, and DNA, are all different agents, to simulate how the system as a whole interacts. This allowed us to ask questions about how different amounts and levels of chemicals in our mixture, which all have different dissociation constants, lead to differences in gene expression, and to address if concentration/dose addition or response addition explain the results we are seeing. In general, we found that due to the low concentration of estrogen receptor, and due to the fact that binding is largely competitive and driven by affinity, that when there are large differences in the affinity of members of a complex mixture, that the chemicals with the most affinity tend to win out, with very little interaction between members of the mixture, meaning the potency tended to reflect those chemicals with the most affinity. We assumed for simplicity that efficacy was the same for all chemicals, which we know is not true, and were primarily interested in changes in potency due to the mixture. Future work will expand the model to also include efficacy estimates. The US Army Chief of Engineers has approved this paper for release. The views presented in this article do not necessarily reflect current or future opinion or policy of the U.S. Army Corps of Engineers.

WE213

Androgenic and Estrogenic Potency-Thresholds in Fish: The HRPT Concept in Ecological Hazard Identification, AOPs, and Cumulative Risk Assessment

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The European Union has recently adopted Criteria and Guidance for the identification of endocrine disrupting chemicals (EDCs), defined as chemicals that produce adverse effects via endocrine mechanisms. Identifying and characterizing toxic hazards based on the manner by which those adverse effects are produced rather than on the nature and likelihood of those adverse effects at relevant exposure levels departs from traditional practice and requires a proper interpretation of the evidence regarding the chemical's ability to produce physiological effect(s) via a specific MoA. The ability of any chemical to produce a physiological effect depends on its pharmacokinetics and the potency by which it acts via the various MoAs that can lead to the particular effect. A chemical's potency for a specific MoA – its mechanistic potency – is determined by two properties: 1) its affinity for the functional components that comprise the MoA, i.e., its specific receptors, enzymes, etc., and 2) it's ability to alter the functional state of those components (activity, activation). Recently an empirical approach was developed for determining the minimum level of mechanistic potency necessary for a chemical to be able to act via a particular MoA in humans, called the Human-Relevant Potency Threshold (HRPT) [1]. Here, we show how the HRPT concept can be extended to analogous mechanistic thresholds in fish using comparative data for human and fish oestrogen and androgen receptor systems. In addition, a practical method is proposed for using taxa-based potency thresholds for the identification of potential ecological hazards through an endocrine mechanism (i.e., is there sufficient mechanistic potency downstream to link to key events in Adverse Outcome Pathways (AOPs). This approach can also be used to assign chemicals to common assessment groups for mixtures risk assessment.

WE214

Human-Relevant Potency Threshold for ER alpha-Agonism

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Potency is a general expression of response per unit of chemical, but it is not single concept that can be applied uniformly to all aspects of chemical effects. Potency for a mode of action (MoA) is conceptually different from potency for an adverse effect; these are measured and interpreted differently. The ability of chemicals to produce physiological effects depends on their pharmacokinetic properties as well as their potency for acting via the various the MoAs underlying the effects. Besides pharmacokinetics, a chemical's ability to produce a physiological effect via a particular MoA depends on its affinity for the functional components (receptors, enzymes, transporters, etc.) that comprise the MoA and its ability to alter their functional state (intrinsic activity or intrinsic efficacy). We refer to the product of affinity and intrinsic efficacy as 'mechanistic potency' to distinguish it from toxicological potency, which refers to the potency by which a chemical produces an adverse effect, and which depends on pharmacokinetics as well as the chemical's ability to act via all of the various modes of action that lead to the particular adverse effect. Mechanistic potency is thus the property that relates to a specific MoA, and which is the requisite property necessary to determine whether a chemical can produce an adverse effect via a particular MoA. We tested the hypothesis that a minimum level of mechanistic potency is required for chemicals to produce physiological effects by a particular MoA in humans by comparing their potencies for the ERα-agonist MoA to data from clinical trials for chemicals with known eostrogenic effects (endogenous hormones and human pharmaceuticals), equivocal estrognic effects (the most potent members of the class of chemicals known as botanical estrogens), and no estrogenic effects (androgens and the least potent botanical estrogens). Potency data from cell-based transcriptional activation assays for human ERα (ERTA) were used to estimate mechanistic potency and the rodent uterotrophic assay (RUA) was selected as an in vivo check on the in vitro potency estimates. Based on these calculations, we proposed a human-relevant potency threshold (HRPT) for ERα agonism of 1E-04 relative to the potency of 17β-estradiol or the pharmaceutical estrogen, 17α-ethinylestradiol.

WE215

Assessment of Endocrine Properties of Bisphenol A in the Environment: A Hypothesis-Testing Weight of Evidence Approach

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Extensive discussion is occurring in the European Union and internationally on how to identify endocrine disruptors (ED). There is general agreement that the evaluation of ED should be based on the WHO/IPCS (2002) definition. According to WHO/IPCS: *"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations."* This definition embodies the key elements of adversity, endocrine mode of action, and a causal link between endocrine activity and adverse effect. The mere existence of an endocrine mode of action (*i.e*., endocrine activity) is not a harmful property, a type of toxic effect, or a hazard. Rather, endocrine activity, if causally linked to an adverse outcome, can potentially lead to a hazard to health or to the environment, particularly after long-term exposure. Defining that there is a causal link between an indication of activity and an adverse effect, particularly one that would be the lead or primary toxic effect most relevant for risk assessment is important. The extensive database for bisphenol A (BPA) was evaluated in a robust weight of evidence (WoE) framework, focusing on specific hypotheses related to disruption of estrogen, androgen, and thyroid hormone pathways. The WoE evaluation included the integration of data from high throughput screens, regulatory guideline studies and published literature, corresponding to levels 1-5 of the OECD conceptual framework. Although a weak estrogenic activity is apparent for BPA among various fish species, the profile of adverse effects that have been measured in BPA-exposed aquatic vertebrates and are used in ecological risk assessments (*i.e.,* effects on survival, growth, development, and reproduction) are not all definitively linked to a weak estrogenic mode of action. The use of bioactivity screens and a consideration of the mechanistic potency of BPA, in relation to endogenous hormones, suggests that the lead mode of action is related to non-specific, systemic toxicity rather than an endocrine specific mode of action.

WE216

Feminization effects on male offspring rats through maternal exposure to flutamide, linuron and dienestrol.

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Flutamide, linuron and dienestrol have been classified as an endocrine disruptors altering endocrine system functions and causing possible harmful effects on the F1 offspring. Flutamide is a nonsteroidal androgen receptor (AR) antagonist and it has been used against prostatic carcinoma. Linuron is an herbicide which acts like an AR antagonist in male rats. Finally, dienestrol is a metabolite of diethylestilbestrol and has been used to treat menopausal symptoms. It acts like an estrogen receptors (ER) agonist. Thus, the aim of this study is to determine feminized physiological traits in male pups due to prenatal and lactation periods through maternal exposure, as well as, to determine the viability and possible toxic effects of the offspring. Female Sprague-Dawley pregnant rats (90 days) were treated daily (gavage) from gestational day (GD) 6, until postnatal day (PND) 21. Each experimental group (5 pregnant females/group) was treated with flutamide at 50, 25, 12.5, 6.2 and 3.1 mg/kg bw/day doses and with linuron at 50, 25, 12.5, 6.2, 3.1 and 1.5 mg/kg bw/day doses. For dienestrol, the doses given were 75, 50, 12.5, 6.2, 3.1, 1.5, 0.75 and 0.37 μg/kg bw/day. The compounds were dissolved in sunflower oil. Control groups with and without vehicle were also included. During weaning, at PND 1, 4, 7, 14 and 21, pup examinations were conducted to assess body weight and feminization endpoints: anogenital distance (AGD) and the presence of nipples (nipple retention (NR)). At PND 21, 4 male pups per litter were anesthetized by intraperitoneal injection, and cryptorchidism was assessed. Testis and liver were removed and weighed. Results showed feminization in all groups of flutamide: decreased AGD, nipple retention and cryptorchidism. Higher concentrations of dienestrol (75 and 50 μg/kg bw/day) caused abortions in all pregnant rats and medium doses (12.5 and 6 μg/kg bw/day) showed some miscarriages. Litters of higher concentrations of linuron (50 and 25 mg/kg bw/day) were not viable. Feminization endpoints can be seen in all flutamide groups and no viability of the litters in linuron and dienestrol in higher-doses groups.

WE217

Estimation of phytoestrogen changes in plants by EDCs using TR-FIA

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Endocrine disrupting chemicals are known to disturb the endocrine system in living organisms. They are widely used around the world for personal care products and are likely to be released into the soil environment. However, there is no international standard for EDCs-specific soil toxicity assessment method unlike human and animal toxicity tests. On the other hand, phytoestrogens are plant-specific xenoestrogens like xenohormones that mimic estrogen activity. Especially, genistein is associated with estrogen receptors in animals, and consequently affects the reproduction of predators. Their effects depend on the environment of the plant and on external stressors. Therefore, we choose genistein, which is a plant-derived substance, to develop as an endpoint of EDCs-specific phytotoxicity. We exposed mung beans to bisphenol A, β-estradiol, and methylparaben known as representative EDCs for 21 days, and then measured the concentration of genistein in mung beans using time-resolved fluoro-immunoassay (TR-FIA). The results showed that the genistein level in mung bean exposed to low concentrations of EDCs increased and as the concentration of substance increase, the genistein decrease. Furthermore, the concentration of genistein in the plants exposed to the highest concentration significantly decreased. The results indicate that the genistein level in plant is more sensitive than the traditional toxic endpoints of plants, such as growth and photosynthesis parameters. Therefore, it is recommended as a pre-screening endpoint for toxicity of EDCs. *This study was funded by the Korea Ministry of Environment (MOE) as the Environmental Health Action Program (1485014458), and the Graduate School of Specialization for managing information of chemical risk.*

WE218

Generation Of NoveL, Integrated and Internationally Harmonised Approaches for Testing Metabolism Disrupting Compounds (GOLIATH)

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The new H2020 project GOLIATH (2019-2024, Grant 825489) focusses on one of the most urgent regulatory needs in EDC testing, namely the lack of methods for testing EDCs that disrupt metabolism – chemicals collectively referred to as ‘metabolism disrupting compounds’ (MDCs). MDCs are natural and anthropogenic chemicals that have the ability to promote metabolic changes that can ultimately result in obesity, diabetes and/or fatty liver in humans. GOLIATH will generate the world’s first integrated approach to testing and assessment (IATA) specifically tailored to MDCs. With a focus on the main cellular targets of metabolic disruption – hepatocytes, pancreatic endocrine cells, myocytes and adipocytes - GOLIATH will develop new methods and optimise existing methods that span the entire adverse outcome pathway (AOP) spectrum, using in silico predictive modelling and high throughput screening, (pre-)validated ready-touse in vitro assays and optimised in vivo toxicity testing guidelines. GOLIATH will provide key information on the endocrine mode of action by which MDCs disrupt metabolic pathways and induce adverse effects on human health by incorporating multi-omics technologies, and translating results from in vitro and in vivo assays to adverse metabolic health outcomes in humans at real life exposures. Given the importance of international acceptance of the developed test methods for regulatory use, GOLIATH will link with ongoing initiatives of the OECD for test method (pre-)validation, IATA and AOP development. With a consortium comprised of world-leading experts in endocrinology, molecular biology, toxicology, epidemiology, test method development, validation and chemical regulation, GOLIATH will be pivotal in the development of an internationally harmonised strategy for testing MDCs, with the ultimate aim of slowing the worldwide rise in metabolic disorders that have reached ‘Goliathan’ proportions.

Benefits of BiER: How Biotransformation and Elimination Rate Related Science Can Improve the Regulation and the Sustainable Use of Chemicals (P)

WE219

The Bioaccumulation Assessment Tool (BAT): Case studies for assessing "data rich" and "data poor" chemicals for regulatory purposes and informing integrated testing strategies

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Persistence, Bioaccumulation and Toxicity (PBT) assessments are carried out by many regulatory programs such as REACh, TSCA, CEPA, and CSCL. These assessments aim to identify the risks posed by chemicals to the environment and human health. The freely-available Bioaccumulation Assessment Tool (BAT) was developed to standardize the collection, evaluation and integration of various lines of evidence (LOE) with available toxicokinetic (TK) information and related B classification criteria. It is a platform designed to aid decision-making using a quantitative weight of evidence approach. The BAT is used to integrate physical-chemical properties and estimates of biotransformation in fish and mammals with measured or estimated bioaccumulation metrics (bioconcentration, bioaccumulation, biomagnification and trophic magnification factors, i.e. BCF, BAF, BMF and TMFs) in aquatic and terrestrial systems. Within the BAT framework is a generic environment with both aquatic and terrestrial foodweb models as well as simulated laboratory models used to estimate these B-metrics. Experimental and in silico LOE are rigorously assessed for reliability, based primarily on OECD guidelines. A summary of the results, including relevance and reliability scoring, are presented in a table as well as in a generated PDF report. This tool is designed to be able to incorporate many types of chemicals and data sources. Both neutral and ionic chemicals can be assessed. We provide an overview of the BAT and demonstrate its application with two case studies. The first example is for a “data rich” chemical for which various LOE exist (e.g. biotransformation rate QSARs, in vitro biotransformation rates, Field BAFs and BMFs, several lab BCFs and various BCF-QSARs). The second case study is a typical “data poor” scenario in which only chemical structure information is available. From chemical structure, relevant physical-chemical property, biotransformation rates are entered into the BAT. Using these data, the tool can generate several LOE and BCF estimates are obtained from QSARs and entered into the BAT. These results can then be used to inform an integrated testing strategy (ITS) to obtain more robust B data if necessary. The BAT is a flexible and transparent framework developed to assess chemicals with varying levels of data availability and properties for their bioaccumulation potential in aquatic and terrestrial organisms.

WE220

Analysis of mammalian toxicokinetic data compilation for use in bioaccumulation assessment

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Elimination half-lives of mammals, including humans, have been proposed as a potential complementary metric to expand the assessment of bioaccumulative properties of a substance to cover air-breathing organisms. In order to explore the possibilities of using such data in bioaccumulation assessment and predictions, a compilation of human and terrestrial mammalian elimination half-life data with almost 3000 data points have been analysed. Regulatory reference cases for bioaccumulation in air-breathing organisms are scarce, meaning a relevant reference data set to derive a threshold is missing. The data suggest that half-lives from repeated dose rat studies generally lead to longer half-lives compared to single dose studies. Consideration of toxicokinetic and toxicodynamic information is expected to significantly improve the understanding of the data and which data are most suitable to use when assessing the bioaccumulation potential in air-breathing organisms. Use of the collected data for benchmarking purposes is suggested. Assessment of the bioaccumulation potential in air-breathing organisms will benefit from an integration of various lines of evidence, and elimination half-life data, if available, should be considered as one of them. Calculation of half-lives is encouraged when performing future ADME studies. Many of the PBT/vPvB assessments finalised in the past with the conclusion “not PBT/vPvB” have not considered bioaccumulation potential in air-breathing organisms. For some of those cases a re-evaluation will be necessary in the future.

WE221

Towards the use of elimination rates in bioaccumulation assessment − current challenges and future needs

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The capacity of chemicals to bioaccumulate in biota is recognized as a critical property that contributes to a chemicals risk. The bioconcentration factor (BCF) reflecting the uptake of a chemical from water and the biomagnification factor (BMF) following dietary uptake in fish remain the preferred metrics in bioaccumulation assessment. Based on the current concept, to derive BCF and BMF, animal tests are mandatory for chemicals exceeding a certain level of hydrophobicity but may turn out as non bioaccumulative due to metabolisation.The test systems are expensive, time consuming and are not suitable for screening purposes. Still, terrestrial bioaccumulation is hardly considered. Recent approaches suggest, to derive BCF and BMF by determining the elimination rate constant (k_2) experimentally while the uptake rate (k_1)is estimated. Following this concept the need for animal tests is reduced if the metabolic contribution to k_2 is quantified in *in vitro* experimentswhile the effect of the other pathways (excretion via urine and feces, and ventilation)are estimated based on existing physico-chemical understanding. Biotransformation often increases elimination and thus reduces the extent to which chemicals accumulate in fish and mammals. Thus, a Tier 1.5 can be introduced between Tier 1 (screening based on physico-chemical data) and Tier 2 (exposure studies with animals) where biotransformation rates (k_m) obtained from *in vitro* tests with fish or mammalian cells are extrapolated to whole organisms and then incorporated into existing chemical mass-balance models to predict a BCF or BMF. Only if this model indicates an increased bioaccumulation a higher-tier vertebrate test would be needed. As such a k_2 based extrapolation model allowing to estimate worst case BCF and BMF values by incorporating *in vitro* $k_m$of different tissues, e.g. gills, liver and gastro intestinal tract from different species, could serve as alternative screening criterion under REACH. This would allow to experimentally cover species differences currently ignored in bioaccumulation regulation. However, uncertainties of this approach remain and will partly be addressed in an UBA project launched early 2019. This poster aims at demonstrating current limitations and future needs for the k_2 based bioaccumulation assessment under REACH from a regulatory agency's perspective.

WE222

Bioaccumulation Regulation: do we need a paradigm change?

K. Goss, Helmholtz centre for environmental research - UFZ / Analytical Environmental Chemistry

The bioaccumulation assessment as it is performed under REACH is based on the BCF in fish using a threshold value of 2000. It appears that this regulation has mostly been the result of a political process. From a scientific perspective questions arise concerning the usefulness of the current approach. This presentation will specifically look at the following issues: 1) Is it correct to assume hindrance in uptake and distribution for superhydrophobic / or large molecules \n as it is done in REACH regulation? 2) Is it sufficient to focus at BCF in fish if we also want to safeguard other vertebrates such as mammals and birds? 3) Is the BCF a suitable metric for a bioaccumulation assessment at all?

WE223

Application of Toxicokinetic Models to Simulate Organic Chemicals in Air-breathing Animals for B assessment

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Bioaccumulation assessment (B) for regulatory purposes has traditionally relied upon data for aquatic organisms (fish) and partitioning properties referenced to water (e.g., octanol-water partition coefficient, K_{OW}). However, emerging regulatory guidance from the European Chemicals Agency (ECHA) includes B screening threshold criteria for air-breathing organisms linked to K_{OW} and the octanol-air partition coefficient (K_{OA}) (e.g., Chapter R11: PBT / vPvB Assessment). Whereas OECD guidance for bioaccumulation testing with fish is available and widely accepted (OECD 305), there is no similar standardized test for air-breathing organisms (e.g., laboratory rats and mice). On the other hand, a wealth of toxicokinetic data has been generated using mammals for other regulatory purposes (e.g., pesticide registration). The objective of this study was to develop and apply one- and multicompartment toxicokinetic models (1-CoTK and PBTK respectively) to simulate the behaviour of organic chemicals in laboratory animals for interpretation in the context of B assessment. The TK models were purposefully developed to be as generic and readily-applicable as possible, based on the understanding that i) assessments are required for both data rich and data poor substances and that ii) the models may be of interest to stakeholders with varying levels of expertise. As a case study, the models were applied to laboratory test data for several organic compounds of interest and their performance assessed. The role of biotransformation in mitigating the bioaccumulation potential of organic compounds in air-breathing animals is particularly evident. Compared to the 1-CoTK model, the PBTK is generally more amenable to simulating and interpreting in vivo laboratory data for B assessment as it explicitly represents different tissues and exchange processes typical of most available testing data rather than operating only at the whole body level. Nevertheless, there are many organic chemicals for which the model performance and output is expected to be very similar between the 1-CoTK and PBTK versions suggesting that the simpler model may be applied with confidence in many scenarios.

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WE224

In silico investigation of hepatic clearance in rodents

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The CEFIC LRI ECO44 project "Integrating Bioaccumulation Assessment Tools for Mammals (iBAT-Mam)" has the aim to integrate experimental data and data generated by *in silico* approaches, to improve mammalian bioaccumulation (B) assessment. Within this context, models based on *in vitro-in vivo* extrapolations (IVIVE) and Quantitative Structure-Activity Relationships (QSAR) are used to maximize the information available from *in vitro* and *in vivo* measurements and to fill *in vivo* biotransformation rate data gaps. This study presents the preliminary results generated from the statistical analysis of *in vitro* data for intrinsic hepatic clearance available in the iBAT-Mam project database. These data were measured for more than 7000 heterogeneous chemicals in rodents by different *in vitro* assays (i.e. S9, hepatocyte, microsomal). Descriptive statistics were applied, such as Multi-Variate Analysis (MVA) e.g. Principal Component Analysis (PCA) and Cluster Analysis (CA), to evaluate the statistical distribution of experimental responses, correlations and to investigate the presence of structural patterns. In addition, preliminary QSAR models were generated using multiple training sets created on the basis of structural similarity investigated by MVA. Theoretical molecular descriptors used as input for MVA and QSAR modelling were generated from canonical SMILES. Good overlap of the structural domain covered by the three diffent assays i.e. S9, hepatocyte, and microsomal, was highlighted by PCA, with a similar distribution of the studied compounds in the space defined by the molecular descriptors. In addition, Cluster Analysis was applied to divide the studied compounds into different structural groups. Finally, QSAR models were generated to predict *in vitro* hapatic clearance measured with multiple assays for different structural groups; particular attention was paid to generate models compliant with OECD guidelines for QSAR models development and validation i.e. effort was made to generate statistically reliable, and externally predictive models with defined structural applicability domains (AD). These results demonstrate that QSAR methods can predict biotransformation rate-related endpoints which are relevant to improve bioaccumulation assessment and to increase the scientific evaluation of hazard and risk that chemicals may pose to humans and the environment.

WE225

Interpreting OECD 305 Dietary Bioaccumulation Tests with the ADME-B Calculator: BCFs, BMFs and Biotransformation Rates

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Current OECD 305 guidelines for conducting bioaccumulation tests include the option to conduct a dietary bioaccumulation test for assessing a chemical's bioaccumulation behaviour. However, the one-compartment toxicokinetic model that is used to analyze the results of dietary bioaccumulation tests is out-of-step with the current bioaccumulation science and experimental practices and information needs for bioaccumulation and risk assessment. This study presents (i) a two-compartment toxicokinetic modeling framework for describing the bioaccumulation of neutral hydrophobic organic chemicals in fish; and (ii) an associated toxicokinetic analysis tool (ADME-B calculator) for the analysis and interpretation of the results from dietary bioaccumulation test in terms of the adsorption, distribution, metabolism and excretion (ADME) of chemicals for the purpose of bioaccumulation (B) assessment. The model framework and ADME-B calculator were applied to the analysis of 229 OECD-305 dietary bioaccumulation tests involving 166 unique organic chemicals. Bioconcentration and biomagnification factors, as well as somatic and intestinal biotransformation rates were derived from this analysis. The findings indicate that the two-compartment fish toxicokinetic model over has the advantage over the one-compartment fish model is that the effect of the exposure pathway on the bioconcentration and biomagnification factor of biotransforming chemicals can be quantitatively taken into account. This is important for the application of a weight-of-evidence based approach to bioaccumulation assessment where information from both aqueous and dietary bioaccumulation tests needs to be interpreted in terms of the chemical's bioaccumulation potential in the environment.

WE226

Biotransformation of chemicals in different species using in vitro metabolism approaches

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Biotransformation of chemicals in different species is not only relevant in bioaccumulation assessment but is an area that requires research. Recently, OECD Test Guidelines (TGs) 319A and 319B were approved for *in vitro* metabolism assessment of chemicals using rainbow trout liver S9 and cryopreserved hepatocytes for application in chemical risk assessment. Although these TGs were developed using rainbow trout, the methodology can be adapted and used across species. Aquatic and terrestrial organisms display a wide range of feeding modes, reproductive strategies and live in variety of environments. For the present study we used liver S9 sub-cellular fractions from a variety of organisms including fish (rainbow and brown trout, Russian sturgeon, bowfin, and alligator gar) and tetrapods (American alligator, rat and human). We compared fish species that inhabit different environments such as the alligator gar that resides in high temperature waters (T > 30ºC) with low DO, and a wide range of salinities to rainbow trout, the reference fish that has been used for *in vivo* and *in vitro* metabolism and bioaccumulation studies. Trout, sturgeon, garfish and alligator were maintained for 2 to 4 weeks under corresponding natural temperature and photoperiod regimes prior to euthanasia and liver S9 preparation. Bowfin were obtained from the field and rat and human liver S9 sub-cellular fractions were acquired from commercial sources. Fish were euthanized and livers perfused with a buffered clearing solution (pH = 7.8), excised, homogenized, and centrifuged to obtain the liver S9 sub-cellular fractions according to the OECD TG 319B. Liver S9 fractions of the different fishes were used to perform metabolism studies with a pharmaceutical (diclofenac), pyrene (PAH), a pesticide and to determine conventional enzyme activities (EROD, GST). Our results indicate that there are differences in metabolism when compared to trout. Alligator gar and bowfin EROD and GST activities were similar to rainbow trout and exhibited significant metabolism of pharmaceuticals such as diclofenac. Alligator (reptile) did not metabolized pyrene as expected. It is interesting to note that ancient species such as alligator gar and bowfin diverged from the teleost evolutionary line prior to the teleost genome duplication (TGD), thus these ancient fishes may possess unique adaptations to cope with xenobiotics in a way that others may not.

WE227
Analyzing complex mixtures for bioaccumulation potential using the trout in vitro metabolism assay (OECD TG 319A and 319B): a case study, fir oil (pine oil) a Natural Complex Substance (NCS).
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Bioaccumulation potential determination using the bioconcentration factor (BCF) is used widely by regulatory agencies (e.g. REACH, ECHA) for the PBT (Persistency, Bioaccumulation and Toxicity) criteria in risk assessment of chemicals. Biotransformation of chemicals is a key component in order to determine if a chemical is enzymatically degraded by organisms in the environment and impacting environmental and human health. We are utilizing the recently approved OECD TG 319A and 319B trout *in vitro* metabolism methodology to assess the bioaccumulative potential of fir (pine) oil. The objectives of the present study are two-fold: a- determine the metabolism of pine oil by assessing biotransformation of 9 main components (alpha-pinene, beta-pinene, camphene, careen, limonene, terpinolene, borneol, borneo acetate, and beta-caryophyllene) b- compare these results with a recent *in vivo* benchmarked fish dietary study of the same fir oil mixture that has been undergoing in McLeod's laboratory at the Stockholm University. In our animal alternative methodology *in vitro* study we are utilizing rainbow trout liver S9 and cryopreserved hepatocytes. The analytical method has been optimized in order to separate and resolved the main components of this mixture for accurate analysis. In addition, individual components have been used to match the identity of the components in the complex mixture of fir oil. These individual components will also be assessed in a subsequent phase of the study for *in vitro* metabolism using the same TG 319A and 319B and compare the results with the behavior of each component in the mixture. The *in vitro* metabolic assay is a powerful tool that can be used to determine BCF of test chemicals and also provides data to build the database information on fragrance materials for fish metabolism and modeling. In addition, this *in vitro* methodology offers the great advantage and flexibility of varying factors at the cellular level in order to obtain a more realistic scenario of the chemicals-enzyme interactions (e.g. inhibition, induction) which is not possible utilizing *in vivo* methodologies.

WE228
Validation of batches of rainbow trout liver S9 sub-cellular fractions (RT-S9) to determine in vitro intrinsic clearance for bioaccumulation assessment
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In vitro-in vivo extrapolation models can be used to predict fish bioaccumulation based on measured *in vitro* biotransformation rates. Two methods to determine *in vitro* intrinsic clearance using hepatocytes and liver S9 sub-cellular fractions (RT-S9) from rainbow trout underwent international validation studies and OECD guidelines have been published. The validation studies showed good transferability and reproducibility of the assays, which were performed on identical batches of biological material obtained from one source. Since these methods are based on primary biological material and not on stable cell lines, and since the biological material is not sourced from a single commercial supplier, variability due to the heterogeneous sources of the biological material must be controlled and minimized. Here we propose a suite of assays to determine phase I and phase II enzyme activities in different batches of RT-S9. Enzyme activities for a total of 9 RT-S9 batches obtained from 4 different sources were characterized. These batches included one batch used in the international validation studies. Model substrates for CYP1A (7-ethoxyresorufin-dealkylation, EROD), uridine diphosphate glucuronosyltransferase (UGT) and glutathione transferase (GST) were applied. Additionally, substrate depletion assays were performed according to the new OECD TG319B with testosterone (CYP3A) and 7-hydroxycoumarin as substrates. The latter was tested with different cofactors to determine sulfotransferase (SULT) and UGT activity. In addition, pyrene (PYR) and cyclohexyl salicylate (CS), two chemicals assessed in the validation studies, were tested. Testosterone depletion, GST- and SULT-activities were similar in the 9 RT-S9 batches tested and differed less than 2-fold. A slightly higher variation was observed for depletion of CS. The highest variations between the batches were found for UGT- and EROD-activities and PYR-depletion. The batches of two commercial suppliers showed comparable activities to the batch used in the international validation study. Based on these data, enzymatic activity ranges are proposed to validate any new batch of trout S9 for key enzymatic activities and, most importantly, to compare new batches directly to the batches used in the international validation studies. This will facilitate comparison of results obtained by different laboratories with the validation exercise and to validate newly generated results obtained with the new OECD guideline.

WE229
Development to measure biotransformation rates of chemicals using hepatopancreas S9 fractions from carp (Cyprinus carpio)
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To rapidly assess bioaccumulation potential of chemical substances, *in vitro* testing methods using hepatocytes or liver S9 fractions from rainbow trout were established recently (OECD TG319A&B) since data regarding biotransformation of chemicals in fish improve significantly the prediction of bioaccumulation potential (BCFs : Bioconcentration factors) in fish. However, the application of this method was substantially limited to rainbow trout. Therefore, there is uncertainty regarding the differences in biotransformation rates among fish species. In this study, we addressed to measure *in vitro* biotransformation rates using carp (*Cyprinus carpio*), which have been used for *in vivo* bioaccumulation assessment such as OECD TG305 in Japan. Through compiling in vitro data of carp, the comparison with *in vivo* data will be possible and it will show the accuracy and the reliability of *in vitro* method. Firstly, we prepared hepatopancreas S9 fractions from carp, because of the easiness to prepare and the difficulty in perfusion and separation of hepatocytes. Then, we measured the Phase I and Phase II enzyme activities and the *in vitro* intrinsic clearance ($CL_{INVITRO}$; mL/min/mg S9 protein) for some chemical substances. We also measured that for liver S9 fractions from rainbow trout and compared these data. As the results, the $CL_{INVITRO}$ values of carp were comparable with that of rainbow trout for tested chemicals and show the usefulness to assess the biotransformation ability of chemicals. However, for liver S9 fractions from rainbow trout, there are large differences in $CL_{INVITRO}$ values between measured values and the previously reported values. Then, it could not be concluded that there are differences among fish species in biotransformation of chemicals. Therefore, further research on species differences and lot to lot differences in biotransformation are needed to be adopted as the testing method by risk assessors.

WE230
SETAC Bioaccumulation Science Interest Group
C. Schlechtriem, Fraunhofer IME / Department Bioaccumulation and Animal Metabolism
.

Achieving Social Equity, Protection of Ecology Systems, and Sustainable Economics (P)

WE231
Describing methods for stakeholder identification, engagement, and empowerment on mining projects.
L. Kapustka, LK Consultancy
In the last three decades, considerable progress has been made in efforts to identify, engage, and empower stakeholders in the management of their environment. Standard procedures have been codified, but all too often, only lip-service is given to stakeholder involvement. It is particularly challenging when Indigenous Peoples have an interest in the proceedings. This is in large part do to the linear thinking of most engineering-based projects. Mining operations typically are in remote areas that often include territories occupied or at least used

by Indigenous Peoples. Their knowledge and values deserve special considerations if the Sustainability Goals of the United Nations are to be addressed meaningfully – Atlas for Mapping Mining to the Sustainable (http://unsdsn.org/wp-content/uploads/2016/11/Mapping_Mining_SDGs_An_Atlas.pdf accessed 15 August 2018). In this presentation, I will trace the interconnectedness of methods for stakeholder engagement and environmental management. This will include details of the ASTM Consensus-Based Environmental Decision-making practice, a comparison with other methods such as iCASS, and illustrate how these could be used to inform decisions toward balancing social equity, ecology, and ecnomics of mining projects.

WE232

Risk profile tables for transparent and holistic decision-making for pesticide authorisation

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Risk assessment and risk management are integral parts of the registration process of plant protection products (PPP). Their functional separation is considered best practice, especially in the European Union. The environmental risk assessment (ERA) investigates whether safe use has been demonstrated for a PPP by meeting its specific protection goals (SPG). In 2016, EFSA guidance was published on the development of SPG, relating them to ecosystem services (ESS) and biodiversity, but not to modern agronomic practices. Indeed, such overarching protection goals require decisions by landscape and risk managers, which should also include the consideration of stakeholders and socio-economic consequences of a given option, including up-scaling from local to global conditions. In this context we propose a risk profile table as tool and structural help. This approach aims at providing a transparent overview of relevant aspects for both the ERA and risk management, and to support the decision making process at the risk manager level. The proposed risk profile table is based on the SPG dimensions as per EFSA (2010), which directly relate to the ERA of a specific product. We propose to add further dimensions, e.g. to inform about risk mitigation or landscape management options, and which can be used to ensure safe use of the product in an agriculturally sound context. \n While the risk profile allows a direct comparison of risks versus benefits, their consequences and includes visualizing the suitable level to handle challenges, the next step is for the applicant and regulators, from risk assessors to risk managers, to test this concept for practical implementation.

The Fate, Effects, and Mitigation of Oil Spills on Aquatic and Marine Environments (P)

WE233

Comparative temperature-dependant toxicity of light oil, intermediate oil and diesel oil WAFs alone and mixed with dispersant: sea urchin bioassays

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Shipping and oil platforms in the North Atlantic and Baltic Sea have been growing during the last years and thus, the risk of oil spills is gaining concern. Different factors such as the type of spilled oil, weather conditions and water temperature can modify the potential toxicity of spill products; So dispersants do, as well. Moreover, dispersants can be toxic themselves and make oil chemicals more available to biota, and though they can contribute to reduce the residence time of the oil in the environment (depending on factors such as the environmental temperature and the oil type) they also cause the concentration of hydrocarbons in water to rise, resulting in enhanced toxicity. Presently, the influence of Finasol OSR52 dispersant on the toxicity of the water accommodated fraction of three oil types, Napthenic North Atlantic (NNA WAF), Intermediate Fuel Oil 180 (IFO180 WAF) and Marine Diesel Oil (MDO WAF) for sea urchin larvae and embryos (*Paracentrotus lividus*) was compared after preparing WAF (oil and oil+Dispersant) at different seawater temperatures (5, 10, 15, 20 and 25°C). Different PAH levels and profiles were recorded in WAF depending on the oil type, the temperature and the presence of dispersant. Developmental alterations and larvae abnormalities were determined, and catalase enzyme activity was analysed in larvae as oxidative stress biomarker. Overall, toxicity was lower for oil WAF than for oil+D WAF at all the studied temperatures. Thus, the presence of dispersant seemingly increased oil toxicity; however, the degree of effect varied with the oil type and the temperature at which WAF was produced.

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WE234

Critical analysis of oil toxicity testing - recommendations and research needs for standardized and site-specific methods

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A review of laboratory studies assessing biological effects of oil products to fish found a diversity of experimental designs, including differences in experimental designs, procedures for preparing test solutions, characterization of test solutions, and end-points measured. The diversity of test methods limits the comparisons of toxicity among studies and among various oil types and weathering states, contributes to uncertainty in estimated toxicity, and may limit their application in oil spill risk assessments. This review demonstrated the need for standardized tests to generate unbiased estimates of toxicity, and the need for research to identify experimental and ecological factors that determine oil toxicity under site-specific environmental conditions. This presentation provides a rationale for developing standard and site-specific oil toxicity test methods, and specific recommendations and research needs to develop such methods for oils with varying properties. Improved assessments reduce the uncertainty in their use in hazard assessments, a major component of environmental risk assessments of oil spills.

WE235

The impact of microbial degradation of oil spills on toxicity to early life stages of marine fish

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Following acute oil spills in the marine environment, microbial communities will metabolize and ultimately mineralize petrogenic oil components. During this process, intermediate metabolites formed are expected to display lower bioaccumulation potential and acute toxicity to marine organisms due to microbial-facilitated incorporation of chemical functional groups resulting in a general decrease in lipophilicity. However, toxicity and characterization of metabolites are poorly studied. The purpose of the present work was to assess toxicity of water-soluble oil components degraded over a three-week period in cold sea water. Low-energy water accommodated fraction (LEWAF) of a weathered crude oil was prepared with nutrient-amended seawater at 5 °C, kept in the dark, and sampled at 0, 10, 14, and 21 days. Samples were extracted with dichloromethane and they were reconstituted back into sea water for toxicity testing. 4-day exposure experiments were conducted for developing cod (*Gadus morhua*) embryos during a critical period of their heart development. After exposure, embryos were kept in clean seawater and observed until 5 days post hatch, and survival, hatching, morphometric deformations and cardiac function were studied. Although reduced larvae sizes were observed after hatch for all treatments, growth rates were not significantly affected. However, developmental deformations and altered cardiac functions were affected in a concentration-dependent manner for WAFs sampled at all time points, i.e. the expected decrease in sublethal toxicity during the biodegradation period was not observed. This implies that either metabolites formed during microbial degradation of water-soluble oil components display equal toxic potential to their parent compounds, or there are other unidentified compounds contributing to toxicity persistent to a 3-week degradation in sea water. In order to properly understand the impact of oil spills on early life stages of fish, efforts need to be made to identify the toxicity driving components of crude oil. And in order to reliably predict impacts of oil spills using fate and effects modelling, metabolites of petrogenic oil components should be included.

WE236

Marine oil spills in the Arctic: quantitative oil spill risk assessment with limited data and high uncertainties

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The Arctic is experiencing increasing levels of maritime traffic, as sea ice continues to retreat due to the global climate change. This development raises concerns about accidental oil spills and their impacts on sensitive Arctic marine ecosystems. In order to be able to develop strategies for proactive risk management, we need methods to assess oil spill-induced risks in the Arctic context. However, limited data and high uncertainties typically hinder oil spill risk assessments in the Arctic. Here we present a novel probabilistic approach for ecological oil spill risk assessment for such cases. We define the oil spill risk (OSR) as the proportion of the population that will die due to the spilled oil within two weeks after an accident, and estimate it by combining information on three components: 1) spreading of oil in ice conditions along maritime routes; 2) spatiotemporally varying species' densities; and 3) species-specific vulnerabilities and sensitivities to oil. We use the Kara Sea, the westernmost of the Siberian arctic seas, as a case study to demonstrate our approach. We included five navigation routes, three seasons, and four oil types in the risk assessment, and the assessment endpoints were three arctic marine mammal species present in the Kara Sea, i.e. polar bear, ringed seal, and walrus. The results show that OSR varies between species, oil types, seasons and maritime routes. For instance, for polar bear and ringed seal, spring has the highest and summer the lowest OSR, whereas with walrus summer ranks before autumn and spring. Oil type has also a clear effect on the risk that species face. The study shows that the risk that potential oil spills pose to the Arctic marine ecosystems depends on many factors. It is also evident that the associated uncertainties are large. However, it is important that these uncertainties are displayed explicitly, if the results are to be used in decision-making. Our method is useful, as it provides quantitative risk estimates with explicitly expressed uncertainty estimates, and offers a relatively simple way to make justifiable risk comparisons over large spatial entities such as maritime routes. However, to be truly comprehensive, the analysis should include also other species groups as well as spatiotemporally varying accident probabilities. Despite these limitations, our study offers valuable information to the assessment and management of oil spills risks in the arctic marine areas.

WE237

Evaluating the impact of a simulated diluted bitumen spill on freshwater zooplankton using limnocorrals

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A number of international and collaborative reports have outlined key research areas to be addressed surrounding the transportation of oil, specifically diluted bitumen (dilbit), around North America's freshwater ecosystems. These reports concluded the need for whole-ecosystem based research evaluating fate and behaviour, toxicity, and remediation of dilbit spills into freshwater. The Boreal Lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) aimed specifically to address this research need. As part of this whole-ecosystem study, the zooplankton communities were monitored for changes in abundance, community composition, and biomass. The study was conducted in an experimental boreal lake at the IISD-Experimental Lakes Area from May 2018 to September 2018. A total of nine 10-metre diameter, 157-m^3, limnocorrals were deployed to an approximate depth of 1.6-m and treated with different volumes of Cold Lake Winter Blend (CLWB) diluted bitumen (dilbit) in a regression-based design (n=7 treatments, n=2 controls). Nominal dilbit:water ratios ranged between 1:100,000 to 1:1,000 (1.6 L to 160 L of dilbit). Zooplankton was sampled at least weekly starting three weeks prior to oil addition and for 11 weeks post-addition. Preliminary data suggests that dilbit has a direct impact on zooplankton communities – densities approached zero in the two greatest treatments with no recovery apparent as of 11 weeks post-addition. In addition, biomass estimates and an analysis of species-specific changes in zooplankton were performed to understand the wider ecological implications following spills of diluted bitumen. These data will help us understand direct and indirect impacts of oil spills on freshwater biota and to inform management and policy strategies for future construction and expansion of oil transportation infrastructure in North America.

WE238

Simulating diluted bitumen spills: Environmental weathering and submergence in model freshwater systems

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The major petroleum product pumped through pipelines in Canada is bitumen, a semi-liquid form of heavy crude that must be diluted with natural gas condensates to facilitate transport. Though diluted bitumen (dilbit) is increasingly transported out of Canada to the global markets, there still exists numerous knowledge gaps in relation to the weathering, fate, behaviour, and environmental effects of a potential dilbit spill into aquatic systems. Here we report the results from a pilot-scale simulated spill of dilbit to freshwater, providing much-needed information in regard to dilbit's behaviour in the environment. The experiment consisted of three outdoor open-air microcosms containing natural lake water and lake sediment, as follows: a high dose (nominal dilbit:water ratio of 1:1000), a low dose (nominal dilbit:water ratio of 1:10,000), and a control (no dilbit), observed over 11 days. Following dilbit application to water, rapid decreases of volatile hydrocarbons in the dilbit slick were observed in the high and low dose treatments within the first 24 hours of the experiment, attributed mainly to evaporation. These changes in chemical composition of residual dilbit coincided with increasing density, viscosity and water uptake. Relatively rapid increases in hydrocarbon concentrations were detected in the water column following the spill, mainly alkylated polycyclic aromatic compounds, which constitute a large proportion of the aromatic fraction of dilbit. Our study provides new in-sights on the environmental fate, behaviour, and risks of dilbit in a freshwater environment. In particular, we are the first to demonstrate the propensity for dilbit to sink under ambient environmental conditions in fresh waters typical of many boreal lakes across the Canadian Shield. In addition to the data on this pilot-scale experiment, preliminary data on our main study conducted in larger (10-m diameter) in-lake mesocosms, will be presented.

WE239

Biological Effects of Chemically Dispersed Crude Oil on Baltic Sea Microbial Communities

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Heavy traffic and transportation of oil makes the Baltic Sea vulnerable to accidental oil spills. Currently mechanical removal is the recommended response method in the Baltic Sea region. However, in some scenarios, mechanical removal can be challenging and some of the spilled oil could remain in the environment. Chemical dispersants are mixtures of surfactants and solvents and are used to treat spilled oil in marine environments. Dispersants break oil slicks into small droplets, which are more readily available for microbial biodegradation. Effects of dispersants on microbial communities and biodegradation rates have not been extensively studied in the Baltic Sea. During this 40-day microcosm study, native microbial communities from the Gulf of Finland were exposed to crude oil, dispersant or their combination. Additional nutrients were not supplied during the study. The experiment was carried out at +10 °C, and samples were collected at days 1, 2, 5, 14 and 40. The amount and expression of genes related to microbial abundance, oil biodegradation and nutrient cycling was assessed by quantitative PCR. High throughput robotics was utilized to study enzymatic activities. The results indicate that microbial response was primarily elevated in samples containing dispersant. Dispersant was degraded almost completely during the study period. However, dispersant application had little effect on overall oil biodegradation. On genetic level, the abundance and expression of alkane hydroxylase gene (*alkB*) was higher in samples with dispersant, whereas the abundance and expression of polycyclic aromatic ring-hydroxylating dioxygenase gene (PAH-RHD) was enhanced also in samples containing crude oil.

WE240

Effects of dispersant on the petroleum hydrocarbon biodegradation and microbial communities in the Baltic Sea

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Dispersants are chemicals used in oil spill response to increase bioavailability and enhance degradation of petroleum hydrocarbons. Although dispersants have been used in several oil spill accidents, there is little information available from the effectiveness of dispersants at the brackish and cold seawater found in the Gulf of Finland and Baltic Sea. This study investigated the effects of dispersant on petroleum hydrocarbon biodegradation rate, degradation gene abundances and microbial communities found at the Baltic Sea.   Microcosm bottle experiments were conducted at 5 °C for 12 days using naphthenic Northern Sea crude oil and dispersant Finasol 51. Seawater used in the experiment was collected from coastal and open sea area of Gulf of Finland and also from coastal area of North Atlantic. In the experiments crude oil was applied as Water Accommodated Fractions (WAF) and dispersant was applied as Chemically Enhanced Water Accommodated Fractions (CE-WAF). Experiments were conducted with diluted and undiluted WAF and CE-WAF. Oil degradation was assessed by analysing the petroleum hydrocarbons with GC-FID at the beginning and end of the experiment. Microbial biodegradation potential was assessed by qPCR targeting PAH and alkane degradation genes. Highest petroleum hydrocarbon degradation was observed in coastal Baltic Sea seawater without dispersant (degradation 92 %), with dispersant addition the degradation was only 5 %. With North Atlantic seawater the degradation was lower, 59 % without dispersant and no overall degradation with dispersant addition. Coastal Baltic Sea seawater showed higher abundance of alkB, PAH and 16SrRNA compared to coastal North Atlantic Sea, which had similar or slightly higher copy numbers compared to open sea Gulf of Finland seawater. With all experiments there was small difference between alkB and 16SrRNA Compared to PAH degradation gene copy numbers, alkB genes were generally 10^3 times greater, indicating a higher immediate potential for alkane degradation. Based on the results, dispersant addition was not observed to increase biodegradation. Microbes associated with coastal seawater were observed to have higher oil degradation gene abundances.

WE241

The importance of primary and ultimate biodegradation of oil compounds in marine fate and effect models

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Biodegradation is an important fate parameter of oil released to the marine environment. Biodegradation rates, based on experimental data, are included for oil compound groups in the OSCAR oil spill model, and for single compounds in the DREAM model for produced water. However, the models do not separate between primary and ultimate biodegradation. We showed in this study that both primary and ultimate biodegradation of oil fractions may be important to determine in relation to different ecotoxicity endpoints of marine organisms. Acute screening toxicity (Microtox^{TM}) was primarily related to primary biodegradation of oil. However, chronic toxicity to developing cod embryos and larva, in particular craniofacial and jaw deformations, were observed mainly unchanged after > 95% primary biodegradation, while ultimate biodegradation was still < 25%. These chronic effects therefore extended beyond primary degradation and were also associated with degradation products or the persistent unresolved complex mixture (UCM) of the oil. In order to relate oil compound biodegradation to different ecotoxicity endpoints, we therefore investigated both primary and ultimate biodegradation rates of selected single components included as naturally occurring substances in the DREAM model, and which were associated with environmental concern (BTEXs, PAHs and phenol/alkylphenols). Reviews of literature data relevant for biodegradation in marine environments revealed a need for revision of the current data in the DREAM model. There was in particular a substantial lack of relevant marine ultimate biodegradation data of PAHs and alkylphenols. Experiments were therefore performed to determine primary and ultimate biodegradation rates of single PAHs and phenol/alkylphenols. The substances were immobilized to hydrophobic adsorbents (Fluortex^{TM}), which were submerged in natural seawater and incubatded at 20°C for up to 2 months. Primary and ultimate biodegradation were determined by gas chromatographic analyses (GC-MS) and as biochemical oxygen demand (BOD), respectively.The biodegradation data were also comparded to previous data from studies of produced and crude oil biodegradation. These data from the current study may be included as part of the fate prediction in the DREAM model. Further studies are required to identify biodegradation metabolites associated with different acute and chronic ecotoxicity endpoints.

WE242

Short-term effects of diesel oil and dispersant on marine microbial communities in the Baltic Sea surface water in coastal areas and open sea

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The Baltic Sea is a geologically and biologically unique sea that is highly vulnerable to environmental hazards. This area is constantly subjected to high inputs of hazardous substances due to anthropogenic activities, and the most emphasized threat is the risk of oil spills. The microbiology of the Baltic Sea has not been extensively studied, and most studies have focused on bacteria, leaving archaea and fungi to less attention. In addition to the natural microbial communities of different parts of the Baltic Sea, the effects of diesel oil, and dispersants applied in case of an oil spill, on the microbial communities is yet to be elucidated. This study focuses on comparing the microbial community structure of the Baltic Sea surface water at three distinct locations; the open sea, pristine archipelago, and putatively oil-contaminated coastal water near an oil refinery. In addition, the short-term effects of diesel oil and dispersant on the microbial community of the three locations were studied during a 72-hour experiment. Next generation Ion Torrent sequencing of bacterial V3-V4 and archaeal V4 regions of 16S rDNA and fungal ITS1 regions was used for a community structure analysis. Quantitative polymerase chain reaction (qPCR) was applied to determine the changes in the copy numbers of bacterial 16S rRNA genes and the two genes associated with microbial diesel degradation, i.e. ring-hydroxylating dioxygenases (RHD) and alkane hydroxylases (AlkB). Assessing the natural diesel degradation potential of the Baltic Sea surface water microbiome is of great importance, and the data generated here can potentially be utilized when developing suitable biological oil spill responses in the Baltic Sea area. Research on the Baltic Sea microbiome and the natural diesel oil degradation potential can also be utilized in predicting the degradation rates in different parts of the sea.

WE243

Biomarker responses in mussels (Mytilus edulis) and amphipods (Gammarus oceanicus) from the White Sea exposed to water-accommodated fraction of marine diesel oil

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The White Sea (northwest Russia) is an Arctic marine ecosystem and therefore potentially very sensitive to oil pollution. Studies on the impacts of oil on local biota has so far been scarce, although nearby areas are expected to be increasing in marine traffic and oil production activities in the future, thus increasing the risk of oil pollution. The blue mussel *Mytilus edulis* and the crustacean amphipod *Gammarus oceanicus* are common and ecologically important species in local coastal habitats of the whole region. For the current study, these species were collected from a pristine region close to the White Sea Biological Station in Kartesh (Chupa Inlet, Kandalaksha Bay) and exposed in the laboratory to a water-accommodated fraction (WAF) of marine diesel oil for 7 days with a semi-static scheme at the temperature of 10ºC and a salinity of 26, corresponding to a typical range of environmental conditions in the area. Concentration of oil polycyclic aromatic compounds (PAH) in the exposure media was measured continuously using the TRIos Enviroflu-HC optical sensor. Before and after the exposure different tissues or whole organisms were measured for a suite of biomarkers and indicators of physiological condition, including antioxidant defence system (ADS) enzymes, oxidative damage (lipid peroxidation), acetylcholinesterase activity, proteasome- and calpain-dependent proteolysis, and changes in total lipid level, lipid class and fatty acids composition.The results show clear effects in a number of the parameters measured in both species, accompanied by bioaccumulation of PAH compounds in the tissues of the organisms. Acknowledgements: The study was supported by bilateral mobility grants from the Academy of Finland (AF), the AF research project OILRES, and IB KarRC RAS state theme No 0221-2017-0050.

WE244

Geno- and cytotoxic effects of crude oils on the mussel Mytilus spp. from the Baltic Sea

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Oil pollution is a widespread and continuous problem in the marine environment. The Baltic Sea is a sensitive ecosystem where restricted water exchange, brackish water and low temperatures increase the risk of pollution induced impacts in marine organisms. Biological effects of crude oil were assessed by exposing mussels (*Mytilus* spp.) to the water accommodated fraction (WAF) of (1) Russian crude oil for 1 and 7 days (temperature 10°C) and after 6 month recovery period in the sea, and (2) Naphtenic North Sea crude oil at different salinities (5.6 and 15) for 1, 7 and 21 days (temperature 5°C). Geno- and cytotoxicity were evaluated in thegills cells using the nuclear abnormalities assay. Frequencies (in ‰) of eight nuclear abnormalities (NAs, per 1000 cells) were examined. Micronuclei (MN), nuclear buds (NB), nuclear buds on filaments (NBf), bi-nucleated erythrocytes with nucleoplasmic bridge (BNb), and blebbed nuclei cells (BL) were used as genotoxicity (∑Gentox) endpoints. Fragmented-apoptotic (FA), bi-nucleated (BN), and cells with 8-shaped nuclei were assessed as cytotoxicity (∑Cytox) endpoints. WAF and chemically enhanced-WAF (CE-WAF) of Russian crude oil resulted in significantly elevated ∑Gentox levels at 7 days of exposure. Mussels deployed back to the sea in cages for a 6-month recovery period showed significantly elevated ∑Gentox and ∑Cytox levels in both WAF and CE-WAF treatments in comparison to control. Naphtenic North Sea crude oil CE-WAF at the salinities of 5.6 and 15 induced significantly elevated levels of ∑Gentox after each exposure time point (1, 7 and 21 days), whereas in the WAF exposed group they were elevated only after 21 days at 5.6. Significant higher levels of ∑Cytox were observed after 1 and 21 days of exposure, and only at 5.6.To assess the multi-biomarker response of crude oil exposure, the ∑Gentox and ∑Cytox results were supported by the Integrated Biomarker Response index (IBR) calculated using a number of biomarkers, including enzymes of the antioxidant defense system, lipid peroxidation, acetylcholinesterase activity, and condition index measured from mussels. Chemical analysis showed that the exposed mussels efficiently accumulated polycyclic aromatic hydrocarbons (PAHs) and the uptake was higher in treatments with a dispersant. Increased PAH solubility at lower salinity resulted in higher PAH concentrations at the salinity of 5.6 compared to 15, having also an effect on biomarker responses.

WE245

Ecotoxicological effects of untreated and chemically dispersed marine fuels on cell and organism level

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Within the framework of the EU project "GRACE", various field and laboratory experiments are carried out to assess the effects of oil spills on selected regional relevant and well-developed model species of the Atlantic Ocean and the Baltic Sea. The marine fuel and crude oil toxicity to aquatic organisms is an important topic in ecotoxicology, as increasing oil consumption, exploration and transport pose a high risk for huge and diffuse oil spills. Besides mechanical oil response option also dispersants are used as a chemical means to combat oil spills at sea. Third-generation chemical dispersants are complex mixtures of surfactants, solvents and other additives which allow the oil to spread more widely in the water body and thus degrade more quickly. One method to assess the risk of different kind of oils and dispersed oils in the GRACE project is the investigation of low energy water-accommodated fractions (LEWAF) and chemically enhanced water-accommodated fractions (CEWAF). In the present study two types of marine fuels, IFO180 and MGO, were tested for their acute toxic and genotoxic potential on different biological levels. The chemical dispersant used was FINASOL OSR 52 (Total). The micronucleus test using the zebrafish liver cell line (ZF-L) to assess the genotoxic potential was performed according to the ISO Guideline 21427-2. To investigate the toxicity on organism level the prolonged Fish Embryo Acute Toxicity Test with *Danio rerio* (OECD 236) was performed. Exposure to fuel WAFs led to typical oil-related malformations in embryos commonly described as blue sac disease. Furthermore, the samples showed a genotoxic potential. Results indicate a higher toxicity for the CEWAF treatment of both oil types, which is in agreement with earlier findings

WE246

Is exposure to chemically-dispersed oil more harmful to early stages of the Northern shrimp Pandalus borealis than mechanically-dispersed oil?

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Oil pollution in the sea is a major environmental concern, which can drastically affect the marine ecosystem. Oil and gas activities is expanding oil search and recovery in the North Sea, and currently extends their search to Northern latitudes, including the Norwegian and Barents Sea [1, 2]. The current and future O&G development, together with increasing shipping after ice reduction, pose an increasing risk for accidental oil spill particularly in the high north ecosystem. Oil spill responses involve trade-offs, including the usage of dispersants. However, Scientific knowledge is needed to support decision-making and it is suggested to use a Net Environmental Benefits Analysis (NEBA) to decide on the best counteractions to mitigate ecological impacts following spill. This is especially demanded for key planktonic larval stages which are the most critical life traits to pollution events. such as oil.\nHere, larval fitness parameters such as survival and feeding rates of the Northern shrimp, Pandalus borealis larvae were measured in a series of exposure experiments where both mechanically and chemicallydispersed oil were used. Longer-term effects on the ontogenetic development of the northern shrimp larvae were also assessed. To mimic realistic exposure to oil spill, an experimental setup with high energy mixing was employed [3]. Oil and dispersant were added to achieve a dispersion with a total hydrocarbon (THC) concentration in the range of 10-20 mg/L, simulating realistic oil spill concentration shortly after a spill [4]. Additional treatments with oil only, dispersant only and a seawater control were included for comparison [5]. Relatively short exposure incubations (from 1h to 24h) were followed by longer recovery times (10 days) to assess both acute- (mortality) and longer-term effects (feeding, development). The overall aim was to assess the sensitivity and resilience of the Northern shrimp larvae to realistic oil spill mitigation responses, to support NEBA and oil responders for deciding the best options to combat oil and mitigate impacts to the marine environment, should a spill occur in the high north regions.

Applications of Bayesian Network Models for Environmental Risk Assessment and Management (P)

WE247

Use of Bayesian network modelling for assessing the weight of evidence of an AOP network for UV radiation

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In this study, we use Bayesian network (BN) methodology to evaluate, refine and quantify a conceptual AOP (Adverse Outcome Pathway) model, which describes the adverse effect of UV-B radiation (a non-chemical stressor). This tentative AOP is a complex network linking two molecular initiating events (MIEs: ROS formation and CPD formation) to two adverse outcomes (AOs: reduced survival and fecundity of arthropodes), through nine potential key events. A tentative AOP structure has been developed based on literature studies, and will be evaluated with experimental data. The experiment, *Daphnia* individuals were exposed to UV radiation in 6 different dose-rates in the range 0 - 0.4 w/m2. All variables (the MIEs, KEs and AOs) were measured with three repeated measurements for each dose-rate. The resulting proposed AOP network consists of 10 potential pathways from the stressor to the second adverse outcome (reduced fecundity). This AOP network will be further developed by BN modelling in three main steps: (1) Evaluation and refinement of the proposed AOP structure: using the structure learning capabilities of BN software to suggest the most important key event relationships (i.e. their causal directions and strengths) based on correlations in the dataset, and refining the AOP model accordingly. (2) Quantification of the refined AOP model: quantifying each selected KER as a conditional probability table based on statistical modelling of the experimental data (e.g., regression analysis of dose-response curves). (3) Sensitivity analysis: ranking the pathways of the AOP network according to the strength of their influence on the adverse outcomes. The approach demonstrated here - the combination of expert knowledge, experimental data and advanced modelling methods (Bayesian networks) - is a promising strategy for data-oriented selection of key AOPs and for the identification of knowledge gaps associated with these AOPs.

WE248

Using AOP Bayesian Networks and Conventional Bayesian Networks to Improve Scenario-Based Risk Assessment and Management

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Bayesian networks are a tool for studying causal chains to predict either outcomes, or the sequence of events that may have caused an outcome. Recently my collaborators and I have demonstrated how adverse outcome pathways can be converted into Bayesian Networks to facilitate the prediction of adverse outcomes using a minimal set of sufficient key events (MINSSKE). Here, I am combining the AOP Bayesian Networks, which operate at a more mechanistic toxicology level, with more conventional Bayesian Networks, that we use for scenario analysis in risk assessments and risk management decisions. Specifically, I will demonstrate how different climate change scenarios will alter the probabilistic risk of an adverse outcome in sensitive species due to some chemical exposure, where the chemical exposure level and route of exposure change as a function of climate change scenarios. I will demonstrate this work using the fungicide prochloraz, our AOP Bayesian Network for steroidogenesis, and common scenarios for climate conditions that may alter the growth conditions of fungus on crop plants.

WE249

Spatial oil spill risk assessment for threatened coastal biota in the northern Baltic Sea: Combining Bayesian networks, oil spill modeling and biodiversity data

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Possible oil accidents pose a risk to marine and coastal biodiversity especially in areas with high maritime traffic density and oil transportation volumes. Spatial oil spill risk assessment can help to manage the risk, for instance, by providing relevant information related to the deployment of oil combating equipment. In this study, we applied a probabilistic method to assess the spatial risk that tanker accidents pose to coastal ecosystems in the northern Baltic Sea. Our approach combined three elements that all had a spatial dimension: (relative) probabilities and other features of tanker accidents in different areas, the stochastic spreading of oil, and the ecological values at stake. We focused on threatened habitat types and species, as they are typically already stressed by other anthropogenic activities, and their recovery can be assumed to be slow and uncertain. We used a Bayesian network (BN) to describe different tanker accident scenarios in a probabilistic manner. The model included six relevant variables needed to describe the accidents, such as location, accident type, spill size, and oil type. The prior distributions for the variables were mainly derived from maritime and port statistics. For the spreading of oil, we used maps calculated with the SpillMod oil spill model that showed the probability of oil to occur in a certain location within 10 days after an accident, given the characteristics of the spill and the prevailing weather conditions. The species and habitat data covered the locations and abundances/areas of threatened species and habitats along the Finnish coastline in the Gulf of Finland and the Archipelago Sea. The results show that spatial oil spill risk varies across the study area, and does not correspond to the frequency of accidents in a given area. In general, the risk is highest in the outer archipelago, and also certain hotspots along the coastline can be identified. Further, the risk is highest for seashore meadows and sandy beaches, which rank high also when species-specific risks are examined. Our study suggests that we should aim for oil spill risk assessments that do not focus only on single factor such as accident probabilities. A major advantage of our approach is the large number of separate oil spill simulations, which are combined by using a BN that offers an explicit way to handle uncertainty related to oil accidents.

WE250

Managing aleatory and epistemic uncertainty in Bayesian Networks

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Bayesian Networks (BN) are probabilistic causal models with applications in risk, decision and data analysis. A BN is a joint probability distribution of nodes and causal links between them. When the nodes in a BN are categorical random variables (which is often the case), this probability distribution is characterized by probabilities or conditional probabilities. These probabilities can express aleatory uncertainty (variability) or epistemic uncertainty (uncertainty due to lack of knowledge). We claim that both types of uncertainty cannot be considered at the same time in BNs, which is a potential problem when there is a need to separate aleatory from epistemic uncertainty in an assessment. We present and compare several alternatives to overcome this issue: 1) Let probabilities in the BN represent aleatory uncertainty only, specify multiple BNs considering epistemic uncertainty in these parameters and evaluate the impact of this uncertainty on the outcome using sensitivity analysis, 2) Specify aleatory uncertainty in the definition of nodes of the BN and use probabilities for epistemic uncertainty only, 3) expand the BN to a Bayesian model which allows for several levels of uncertainty and uses the probability on relative frequency approach to treat epistemic and aleatory uncertainty, and 4) Use the BN for aleatory uncertainty only and consider epistemic uncertainty by bounds on the probabilities (Credal Net). These alternatives are valid and coherent alternatives to manage aleatory and epistemic uncertainty in applications of data or expert-driven Bayesian Networks for environmental impact or risk assessment.

Examining Behavioural Effects of Chemical Contaminants and Other Stressors on Behaviour, Ecology and Evolution of Wildlife (P)

WE252

Behavioural and ecological responses to chemical pollution

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Humans have brought about unprecedented changes to environments worldwide. For many species, behavioural adjustments represent the first response to altered conditions. Such behavioural modifications can potentially improve an organism's prospects of surviving and reproducing in a rapidly changing world. However, not all behavioural responses are beneficial. Human-altered conditions, including the discharge of anthropogenic chemicals into the environment, can undermine the reliability of sexual signals used by animals to assess potential suitors. Environmental pollutants can also impair sensory systems or interfere with physiological processes needed to mount an appropriate behavioural response. An understanding of behaviour could therefore be important in helping to explain why some species are able to survive, or even flourish, under human altered conditions, while others flounder.

WE253

Interaction between carbamazepine toxicity and food limitation in an artificial indoor stream experiment

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In chronic toxicity tests, single organisms are commonly exposed to a single contaminant under low-stress laboratory conditions. As those experiments reduce the complexity of natural habitats and potentially the sensitivity of organisms, we exposed three aquatic invertebrates to the antiepileptic drug carbamazepine (CBZ) in a multiple stress, higher tier test. The non-biting midge *Chironomus riparius*, the blackworm *Lumbriculus variegatus*, and the New Zealand mud snail *Potamopyrgus antipodarum* were exposed to two concentrations of CBZ (80 and 400 µg/L) with and without the addition of the herbicide terbutryn (TBY, 6 µg/L) in a 32-day artificial indoor stream experiment. TBY was added as a second chemical stressor to reduce the biofilm biomass in the streams and, hence, induce food limitation. Data were analyzed by an analysis of covariance (ANCOVA) with TBY as a covariate. *C. riparius, P. antipodarum,* and in tendencies *L. variegatus* were affected by CBZ in the artificial indoor streams at concentrations of 10 to more than 25 times lower than in previous laboratory studies. *P. antipodarum* was the only organism affected by TBY as a single factor, which significantly reduced the biofilm biomass. The addition of TBY caused a decrease in emergence of *C. riparius* at 80 µg CBZ/L but an interaction between CBZ and TBY was not present according to the ANCOVA. All three organisms were differently affected by CBZ and the multiple stressors in this experiment according to its ecological niche. The environmental hazard for CBZ can be considered as low due to the high effect concentrations of CBZ. Nevertheless, the difference in sensitivity between laboratory and indoor stream experiments by a factor of more than 25 for *P. antipodarum* give reason for concern about a potential underestimation of risk for other, more toxic contaminants.
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WE254

Impacts of water pH on the toxicity of fluoxetine on Daphnia magna: a multi-generational study

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Fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), has been widely prescribed as anti-depressant. While FLX has been frequently detected in surface waters, sediments and biota, only limited information is available on its *in vivo* toxicity, particularly in invertebrates, under changing water pH. In the present study, the ecotoxicity of FLX (pK_a = 9.8) was investigated in different pH levels (6.8, 8.3, and 9.2) using *Daphnia magna*. Daphnia neonates ((F0) were exposed to 0, solvent control, 31.3, 62.5, 125, 250, 500, or 1000 µg/L for 48 hr and its effects on survivals (EC20, 50 and 75) were determined. Moreover, Daphnia neonates (The first brood daphnids from the chronic tests were discarded and the second brood daphnids (F1) were employed for the multigenerational acute test within 12 hours. F1 neonates were further exposed to 0, solvent control, 31.3, 62.5, 125, 250, or 500 µg/L for 48 hr and their survivals (EC20, 50 and 75) were determined. F0 juveniles exposed to 0 or 55.6 µg/L at 10 d were measured for MDA. After 48 hr of F0 and F1 exposure, the EC50 significantly decreased ($p < 0.05$) at 6.17 µg/L of FLX at all tested pHs. Moreover, after 21 d F0 exposure, the reproduction showed an increasing trend until 55.6 µg/L and significantly decreased at 166.7 µg/L at pH 9.2. F1 neonates showed significantly decreased body length after 21 d exposure to 2.06, 6.17, 18.5 and 55.6 µg/L at pH 9.2. Malformation of F1 daphnids development was also observed at 2.06 µg/L to 500 µg/L. MDA concentration was also demonstrated increasing trends in all pHs and significantly increased at 55.6 µg/L at pH 6.8 and 9.2. Our observation clearly indicates that survival, reproduction, and growth performance in aquatic invertebrate can be adversely affected by FLX and the toxicity increases as water pH increases. A greater amount of uncharged ions from FLX has increased Reactive Oxidative Stress (ROS), MDA, by the ionic speciation at higher pH. Increased MDA concentration affected the mortality of F0 daphnids. Increased F1 daphnids population demonstrated significantly greater toxicity. As a result, the toxicity of FLX has increased acutely, chronically, and multigenerational effects by increasing pH levels. Consequences of longer-term exposure over multi-generations warrant further investigation.

WE255

Evaluating slow-release implants for manipulating pharmaceutical exposures in wildlife

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Recently, scientists have advocated to "scale-up" studies measuring the effects of pharmaceutical pollutants in the wild. Whole-lake experiments are a gold standard for ecotoxicologists looking to assess the effects of chemical contaminants, such as pharmaceuticals, in aquatic ecosystems. However, access to such lakes is limited, exposures can be costly, and they can have potentially long-lasting effects on the exposed environment. Here, we present a method for manipulating internal pharmaceutical exposures in aquatic wildlife. We evaluate the used of two fat-based pharmaceutical carriers (coconut oil and vegetable shortening) for delivering an internal dose of the behaviour-modifying pharmaceutical oxazepam. We implanted roach (Rutilus rutilus) with implants containing a high (50 µg/g), low (25 µg/g), or control (0 µg/g) concentration of oxazepam in the implant. We then measured oxazepam uptake in four tissues (plasma, muscle, liver, and brain) over a month. The two carriers released oxazepam differently: coconut oil delivered a more consistent dose across time, while vegetable shortening released oxazepam rapidly at the start of the exposure period. For both carriers and treatments, brain and liver tissues contained the most oxazepam, while plasma and muscle contained the least. Overall, the method is a promising technique for conducting controlled manipulations of pharmaceuticals in fish. We compare our findings to tissue-specific oxazepam uptake reported in previous waterborne exposure studies, and discuss potential mechanisms (e.g., diffusion) for the differential release of oxazepam from our two fat-based carriers.

WE256

Sublethal behavioral responses of two common larval fish models to Prynesium parvum along a nutrient stoichiometry gradient

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The magnitude, frequency and duration harmful algae blooms (HABs) appear to be increasing on a global scale. These HAB events result in diverse water quality challenges such as impairment of recreational uses, reduced aesthetics, decreased drinking water quality, lower dissolved oxygen concentrations, and health and ecological risks following the production of toxins. *Prymnesium parvum* causes major kills each year around the world, resulting in appreciable economic losses. Unfortunately, sublethal effects of this emerging threat to water quality are largely unstudied. In the present study, we examined growth and sublethal toxicity of *P. parvum* across a nutrient stoichiometry (N:P) gradient (1:1, 4:1, 8:1, 16:1, 32:1, 64:1, 128:1) while maintaining temperature and salinity conditions representative of conditions associated with inland HABs. Following previously reported methods from our laboratory, we examined acute and sublethal behavioral responses of the fathead minnow and zebrafish models during stationary growth conditions. These toxicity bioassays employed embryo-larval stages and followed standardized experimental guidelines. For behavioral analyses, zebrafish embryos and fathead minnow larvae were exposed for 48 h to dilutions of each N:P treatment level and then were observed with a digital behavioral analysis system.

These analyses included photomotor responses, distance travelled, and stimulatory, refractory and cruising locomotor activity. Our observations identify the importance of understanding sublethal responses to *P. parvum* and demonstrate the usefulness of behavioral responses for HAB events.

WE257

Comparative Behavioral Toxicology in Zebrafish: Individual and Mixture Responses to Nicotine and Bupropion

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Pharmaceuticals are a major group of contaminants of emerging concern that have been detected in surface waters worldwide, yet traditional toxicological endpoints (e.g., mortality, growth) may not capture important mechanism of action (MOA) relevant responses of these chemicals in aquatic biota. Because several pharmaceutical compounds (e.g., antidepressants) are specifically designed to modify behaviors in humans, behavioral responses of non-target species must be considered. Unfortunately, data is routinely lacking on specific behavioral perturbations associated with therapeutic targets in fish species. Nicotine and bupropion are two common pharmaceutical contaminants that differentially interact with nicotinic acetylcholine receptors (nAChRs), and elicit behavioral effects in mammals. Therefore, the current study followed standardized guidelines to identify and compare larval zebrafish behavioral responses between nicotine and bupropion after 96 h exposure. A binary mixture study was then conducted with both nicotine and bupropion following previously published methods. Each compound produced locomotor and photomotor effects in larval zebrafish at levels that were two to four orders of magnitude lower than LC50 values. Nicotine, a nAChR agonist, produced stimulatory responses whereby larval activity dramatically increased at bursting speed thresholds, but bupropion, a nAChR antagonist, elicited refractory responses were larval activity decreased at all effected endpoints. With the mixture study at the 1% LC50 concentration for both compounds, nicotine bursting activity was reduced, and the activity was no longer significantly different from the control. Additionally, behavior was affected significantly at the lowest treatment level of these mixtures (0.25% LC50 nicotine and bupropion). These results indicate that behavioral responses can be useful to examine physiological responses associated with pharmaceutical MOAs at concentrations far below those eliciting mortality. These observations further suggest that behaviors can be leveraged to investigate interactive effects of aquatic contaminants on non-target aquatic species.

WE258

Effects of nanoplastics on fish embryos

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Small plastic particles have increasingly been detected in aquatic biota, from zooplankton to fish, raising concern for potential effects on aquatic organisms. Once in the environment plastic debris can be fragmented to smaller-sized plastics, including microplastics and nanoplastics. Although an increasing number of studies have been assessing the effects of microplastics to fish, the data concerning nanoplastics can be considered scarce. This study was design to assess the effects of nanoplastics on zebrafish embryos development, swimming behaviour, response to stress and biochemical endpoints associated with oxidative status and energy metabolism. Considering that in the environment organisms are exposed to plastic particles and other environmental contaminants, this study also aimed to assess the role of nanoplastics on the toxicity of other environmental contaminants, to evaluate their protential trojan horse effect/ protective effects.

WE259

Impact of the agricultural pollutant 17β-trenbolone on sequential male mate choice in the guppy (Poecilia reticulata)

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Endocrine-disrupting chemicals (EDCs) are of major concern due to their capacity to perturb hormone systems of wildlife at minute exposure concentrations. In this regard, a leading source of EDCs in the environment is run-off of veterinary pharmaceuticals used in agriculture. This includes hormonal growth promotants (HGPs), which are administered to beef livestock in agricultural operations worldwide. Among the most biologically active HGPs is the androgenic steroid 17β-trenbolone, which has repeatedly been detected at low nanograms per litre concentrations in surface waters. In standard reproductive trials (i.e. a male-female pair), recent research has revealed that exposure to 17β-trenbolone can alter mating behaviour in fish. However, in reality, natural mating systems are complex and very little is known about the potential for chemical pollutants, in general, to disrupt behaviour under more complex and ecologically realistic reproductive scenarios, such as sequential mate choice. To address this, we exposed wild-caught adult male guppies to a field-realistic level of 17β-trenbolone (average measured concentration: 2 ng/L) for 21 days and compared the response of exposed and unexposed males to sequentially presented large and small females. Due to a positive size-fecundity relationship, larger females often represent better reproductive value and are generally preferred by males. In the first presentation, regardless of exposure, males demonstrated a preference for larger females by courting these females more often. Further, regardless of female size, exposure was associated with a marginally non-significant increase in male sneaking behaviour. In the second presentation, males from both exposure treatments again demonstrated a preference for greater female size in terms of both courting and sneaking behaviour. Furthermore, exposed fish again performed more frequent sneaking behaviour towards females. We found evidence for sequential male choice, although this was not disrupted by exposure. This is because, regardless of exposure, males 'traded down' by significantly reducing the frequency of their courtship behaviour towards small females if they had previously encountered a large female. Considering the pivotal role that mate choice mechanisms play in population dynamics and broader evolutionary processes, this study highlights the need for a greater understanding of potential impacts of chemical contaminants on sexual selection in wildlife.

WE260

Assessing population-relevant behavior patterns in free-swimming groups of larval zebrafish (Danio rerio)

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In order to help identify potential population-relevance of neurotoxic effects on embryos (as revealed in photomotor or locomotor response assays), zebrafish (*Danio rerio*) embryos were exposed to sublethal concentrations of fluoxetine in a 35 d early-life stage test augmented with behavioral assessments. As a known selective serotonin re-uptake inhibitor (SSRI), fluoxetine was chosen as a first model substance. Behavioral analyses of multiple free-swimming fish, however, posed significant technical difficulties, even more so since the zebrafish juveniles observed were very small and not yet fully pigmented. Nevertheless, a promising protocol using groups of 20 fish could be developed to demonstrate more natural behavior patterns than in tests with isolated individuals. A relatively large volume of 8 liters was chosen to allow for natural swarm behavior despite experimental constraints; the large volume also provided ample room for the fish to display spatial preference. Since the swimming tracks of individual fish inevitably cross the tracks of other individuals, individual-based analyses (e.g. of swimming speeds and patterns) had to be abandoned, and the analysis was focused on the distribution of the shoal over time. Manual tracking allowed the analysis [1] of the relative habituation time after stress (novel tank test), [2] of the average distance kept to a potential threat (predator response test) or [3] of the feeding behavior. First results show a clear concentration dependence of all of these endpoints; however, the interpretation of the result required careful consideration of each response since the observations did not allow a simple categorization into "anxiogenic" or "anxiolytic". In some occasions, upon first glance, results even seemed contradictory. Besides the aforementioned behavioral paradigms and the classic early-life stage growth and survival metrics, it should also be possible to quantify swarm coherence and compactness as additional endpoints once the fish can be reliably identified by an appropriate image analysis software.

WE261

Dilute levels of a benzodiazepine (oxazepam) alters important behavioural traits in a common planktivorous fish, (Rutilus rutilus)

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Environmental pollution by pharmaceuticals is increasingly recognised as a major threat to aquatic ecosystems worldwide. A complex mix of pharmaceuticals enters waterways via treated wastewater effluent and many remain biochemically active after they reach aquatic systems. However, to date very little is known about the ecological effects that might arise following pharmaceutical contamination of aquatic environments. One group of particular concern is behaviourally modifying pharmaceuticals because seemingly minor changes in behaviour can generate pronounced ecological consequences. Here we show that a benzodiazepine anxiolytic drug (*oxazepam*) alters key behavioural traits in wild roach *(Rutilus rutilus)* at concentrations similar to those encountered in effluent dominated surface waters. Roach exposed to water with high concentrations (280 $\mu g\ l^{-1}$) exhibited increased boldness, while roach in the low treatment (0.84 $\mu g\ l^{-1}$) became bolder and more active compared to control fish. Our results reinforces the notion that anxiolytic drugs could be affecting fish behaviour in natural systems, emphasizing the need for further research on ecological effects of pharmaceuticals in aquatic systems and the development of new tools to incorporate ecologically relevant behavioural endpoints into ecotoxicological risk-assessment.

WE262

Behavioural effects of temperature, predation-risk and anxiolytic exposure on the European perch (Perca fluviatilis)

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With the ability to resist biodegradation and exert therapeutic effects at low concentrations, pharmaceutical contaminants have become environmental stressors for wildlife. In fact, more than 600 pharmaceutical substances have now been detected in the environment worldwide. Despite increasing research interest in understanding how wildlife responds to pharmaceutical contaminants, the synergistic fitness effects of pharmaceuticals and their interaction with other biotic (e.g. temperature) and biotic (e.g. predation risk) stressors remain unclear. In particular, in aquatic environments, changing temperature due to anthropogenic activity (e.g. seasonal extremes due to climate change), impacts ecosystems at multiple levels of biological organisation. One pharmaceutical contaminant of particular concern is the anxiolytic oxazepam, a psychoactive pharmaceutical that is frequently detected in surface waters globally. Here, using a multi-stressor approach, we investigated effects of 7-day oxazepam exposure (6.5 μg/L) on tendency to shoal (i.e. sociability) in juvenile European perch (*Perca fluviatilis*). The multi-stressor approach was achieved by exposing perch to oxazepam at two temperatures (10 °C and 18 °C), and at two predation risk regimes—generated using chemical cues from the northern pike (*Esox lucius*). Forming shoals provides protection from predation and improves foraging efficiency through information transfer and, if disrupted, has potential to impact the fitness of exposed animals. A standard sociability assay was used to record the amount of time spent near a shoal of size-matched conspecifics. We found that 7-day exposure resulted in a successful uptake of the drug from the water, i.e., oxazepam was measured in perch muscle tissue at 50 ± 17 ng/g (mean ± SD). Results from the sociability assay will be presented and further discussed. Overall, our multi-stressor study is the first to uncover if, and how, exposure to oxazepam alters sociability-related behaviours in wild-caught juvenile fish, and if these alterations are modified by water temperature and perceived predations risk.

WE263
Assessing predator-prey interactions in a chemically altered aquatic environment: The effects of DDT on Xenopus laevis foraging behaviour
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Behavioural assays are used as a tool to understand ecotoxicological effects on organism functioning, however, this is often not applied in an ecologically relevant context. It is essential to assess the effect of chemical contaminants on behaviours relating to fitness and trophic interactions, thus predator-prey interactions must be incorporated in order to create appropriate hazard risk assessments. Dichlorodiphenyltrichloroethane (DDT) is a controlled substance but is still regularly used as a form of vector control. There is little explicit information on the effect of DDT on animal behaviour and the consequent effects upon trophic interactions. This study uses a 3 x 2 factorial design to assess the interactions of predatory behavioural responses of *Xenopus laevis* towards a vector prey (*Culex* sp. larvae) towards visual-hydromechanical and olfactory prey cues, with regards to factors "DDT exposure" and "prey cue". We also assess the behavioural responses of mosquito larvae when supplied with no threat cue and predator threat cues when exposed to 2 μg/L and 20 μg/L DDT. There was a significant "DDT exposure" x "prey cue" interaction wherein DDT significantly decreased the foraging behaviour of *X. laevis* towards visual-hydromechanical prey cues, however there was no effect of DDT upon *X. laevis* response to olfactory prey cues. Dichlorodiphenyltrichloroethane exposure caused mosquito larvae to appear hyperactive moving significantly more; however there was no difference between DDT concentrations. Mosquito larvae anti-predator response was significantly dampened when exposed to 2 μg/L DDT, however when exposed to 20 μg/L the anti-predator responses were not impaired. Our results indicate a complex interplay in trophic interactions under DDT exposure, wherein effects are mediated depending on species and concentration. There are possible implications regarding reduced anti-predator behaviour in the prey species but also reduced foraging capacity in the predator, therein it is likely that DDT exposure can cause changes in ecosystem energy pathways, resulting in bioaccumulation in higher trophic levels. We demonstrate that in order to quantify effects of pesticides upon trophic interactions it is necessary to consider ecologically relevant behaviours of both predator and prey species.

WE264
Could behavioural disturbances be linked to density depletion of the ragworm Hediste diversicolor populations?
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Evaluating the health status of estuarine areas is a difficult task as they are impacted by mixture of contaminants. Chemical approaches are classically combined to biological and ecological indicators. However, chemical analyses are costly and some contaminants are not yet accessible to analyze. This work aims to demonstrate in *Hediste diversicolor*, a sentinel species of most estuaries, the key role of behavioral biomarkers which may be at the individual level the central link between sub-individual response and population changes in the field. Acetylcholinesterase (AChE), a core biomarker of neurotoxicity, was chosen for its ecological relevance as its inhibition observed in laboratory or field studies can lead to behavioral impairments in organisms exposed to a wide range of contaminants. The sampling site was located on the largest mudflat of the polyhaline domain of the Loire estuary ("Les Brillantes"). Two areas were selected in the upper intertidal (Site 1) and in the middle intertidal (Site 2) zones of the mudflat. Two sampling campaigns were conducted in May 2013 and February 2014, respectively. AChE activity was determined in worms from each site. Burrowing behavior was studied by placing worms on sediment of their site of origin and recording their position every 2 minutes during 30 minutes. Additionally, automated behavioral tests were carried out on worms with the Multifreshwater Biomonitor® (MFB®) allowing the identification of *H. diversicolor* movements (undulation, head movements and feeding activity). Density was determined sorting and counting worms from four sediment samples randomly collected at low tide from both areas (Site 1 and Site 2). The results showed a lower AChE activity, behavioral impairments (burrowing kinetics, body undulation, feeding activity) and a lower density for the worms from Site 2 compared to Site 1. A Spearman positive correlation was determined between AChE activity and MFB results suggesting a link between behavior impairments and AChE activity inhibition. These results allow a first attempt to propose three steps of an adverse outcome pathway from AChE inhibition (molecular interaction), behavior alteration (organism response) leading to density decrease (population response) in *H. diversicolor*. These results bring direct empirical field evidence demonstrating the sensitivity and the central key role of behavioral biomarkers in *H. diversicolor* as biomonitors of estuarine health status assessment.

WE265
Impact of the pharmaceutical sotalol on Gammarus pulex swimming behaviour
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Pharmaceuticals in surface waters have become an environmental concern in recent years. Although acute toxicity does not seem to be an immediate issue the impact of chronic exposure of especially invertebrates to these compounds is poorly understood. In addition, an increasing body of evidence becomes available showing that chronic exposure to pharmaceuticals results in behavioural changes. In order to test the potential impact on aquatic invertebrate behaviour we exposed *Gammarus pulex* individuals to sotalol, a beta-blocker frequently occurring in Dutch surface water monitoring schemes. The amphipods were exposed for 7 days to concentrations ranging from 0.03 to 10 ug/L and were subsequently released in a custom-made flow-through device. Flow velocity in the device could be varied and the velocity that proved to be too strong for the Gammarids, i.e. washing the animal out of the tube, was taken as the final endpoint. *Gammarus* swimming ability decreased at increasing sotalol concentrations with the highest impact on swimming ability observed in the 1 ug/L treatment level. Interestingly, no monotonous response could be observed since swimming ability increased again at higher test concentrations, resulting in an a-typical 'arch-shaped' effect curve. Although these results are in line with previous findings for carbamazepine, ibuprofen and fluoxetine (De Lange et al., 2006) it is not yet clear what the mechanistic explanation or ecological consequences for this phenomenon are.

WE266
Development of high-throughput assays to assess swimming behaviours in Artemia franciscana
S.A. Kohler, University of Portsmouth / Aquatic Biology; A. Ford, University of Portsmouth / Biological Sciences; M.O. Parker, University of Portsmouth
Technological advancements have allowed for a rapid expansion in the use of behavioural endpoints in ecotoxicology. However, the lack of standardisation poses the largest drawback in the widespread use of behavioural assays in environmental risk assessment. It has been shown that there is a trade-off between allowing 'space to behave' and reducing arena size to increase high-throughput analysis for your chosen species. Furthermore, some behaviours are complex and require careful experimental design and vast amounts of data on the baseline unconditioned behaviours of your test organism to allow for appropriate interpretation of results. For example, alterations in phototaxis behaviours may be interpreted as scototaxis, representing an anti-anxiety behaviour. Moreover, these responses have been found to differ between seemingly similar species. In this study we optimised a simple swimming behavioural assay using the brine shrimp *Artemia franciscana*. Sudden transition to light was used as a stimulus to initiate a behavioural response. Multiple parameters were measured using a DanioVision

observation chamber with EthoVision tracking software. Significant differences (P< 0.05) were observed between the swimming speed and escape response to light between arena sizes. *A. franciscana* showed a greater mean velocity when in a larger arena. Some reports in the literature suggest that *Artemia spp* can differ in their phototactic behaviours depending on light intensity. In this study we assessed the effects of low and high lux on swimming behaviours and phototaxis. However, no significant differences (P>0.05) were observed in either endpoint between light intensities. Subsequently, we conducted a fluoxetine exposure (0.001-1μg/L) over 1hour, 1 day and 1 week. Significant effects on velocity were observed after 24 hours of exposure (P>0.05) but not after 1 hour or 1 week. Further experiments to test the repeatability of this experiment are ongoing. The use of artemia in high-throughput behavioural assays seems promising to support environmental risk assessment

WE267

Effects of the antidepressant Venlafaxine on the feeding rate and behaviour of the freshwater amphipod Gammarus pulex

G. Consolandi, University of Portsmouth; A. Ford, University of Portsmouth / Biological Sciences; M. Bloor, University of Portsmouth / School of Earth and Environmental Science

In the last decade the prescribing of antidepressants has increased dramatically. Venlafaxine (VEN) is one of the most commonly detected antidepressants in European streams. Little is known about the potential impact of VEN on non-target aquatic organisms at enviromentally realistic concentrations but it is hypothesised to interfere with behaviour and feeding. We aim to identify if the behaviour and/or feeding rate of *Gammarus pulex* a sensitive freshwater macroinvertebrate was altered when exposed to environmentally realistic concentrations of VEN for 7 days. Specimens of *Gammarus pulex* were kept for 14 days at 15?C under a 12:12 h light:dark cycle. After a 48 h starvation period they were exposed for 7 days to 3 different concentrations of VEN (20 ngl^{-1}, 2 μgl^{-1} and 20 μgl^{-1}). To evaluate the possible alteration on the feeding rate (FR), *Alnus glutinosa* leaves were cut into discs, dried and weighted. The leaf discs were then conditioned in stream water for 14 days, after which time each disc was photographed and given to the organisms. Each organism was provided with 2 discs at a time, that were replaced every 2 - 3 days. The leaves were then re-photographed, dried and weighted again. The before and after photographs were analysed to determine the leaf area consumed, whereas changes in the leaf disc weight were used to calculate the consumed leaf mass. To quantify possible changes in the swimming velocity, *Gammarus* pulex behaviour was recorded after 24 h and 7 days by using a DanioVision™ observation chamber. Data were analysed with a linear mixed effects model (p=< 0.05). *Gammarus pulex* FR was not altered. There was no significant difference between the concentrations in either the consumed leaf mass (F=0.764, p=0.519) or the leaf area consumed (F=2.610, p=0.060). The interaction between treatments and time was not significant (F=0.726, p=0.630; F=1.884, p=0.090). However there was a significant overall change in FR over the length of the experiment, but unrelated to the concentration tested (F=14.742, p=0.000; F=62.221, p=0.000). Alterations in velocity were not significant between the concentrations (F=0.934, p=0.430). A significant interaction was measured between concentration and exposure time (F=26.025, p=0.000), meaning that the organisms velocity was different across the concentrations after 24 h and 7 days. Overall this study showed that VEN may affect *Gammarus pulex* behaviour when exposed to environmentally realistic concentrations for 7 days.

WE268

Behavioural and proteomic responses in Asiatic clams Corbicula fluminea, exposed to crude oil in outdoor artificial rivers, within a multistress context.

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Oil industry is a source of disturbance in aquatic ecosystems. Relevant, efficient, and reliable monitoring is essential and requires the use of adapted and specific tools. Robust, autonomous and working by remote control, HFNI (High Frequency Non-Invasive) Valvometry is one possible solution to improve management in natural systems. Its principle is to monitor online, 24/7, behaviour and different life history traits (restlessness, biological rhythms, growth, spawning, mortality…) of abandoned bivalve mollusks. Through distant access, data are automatically readable on the web and allow monitoring them in near real time to research online changes in water quality. To develop and value this biomonitoring instrument in the oil and gas industry it is necessary, to highlight if behavioural responses to crude oil are reliable and discriminating in presence of additional stressors and to explore the relationship between behavioural signals and underlying cellular responses. Looking for basic unifying principles, the behaviour of *Corbicula fluminea* was studied in a multistress context by comparing and coupling the disturbances caused by a crude oil exposure (400 μg/L) in presence and/or absence of noise pollution (cargo noise), barium (100 μg/L) and burst of turbidity. Experiments were conducted in outdoor open-stream mesocosm subjected to natural variations (Artificial Rivers, Lacq, France, TOTAL; 1 month of acclimation and reference, 10 days of exposure, 1 month of recovery; no death). The behavioural response to crude oil was characterized by a decrease of valve opening amplitude and a decrease of valve opening duration. The characteristic behavioral response of bivalves to crude oil exposure was maintained in presence of additional stresses. Furthermore, the proteomic response and the aromatic hydrocarbon bioaccumulation (which remained in the low range) were investigated in gills and adductors muscles of oil-exposed clams. We will discuss the main results and we will conclude that the study of bivalve mollusks behaviour by HFNI Valvometry confirms its biomonitoring potential for the oil and gas industry.

WE269

Analysing zooplankton swimming bahviour with robots and data science

R. Wolf, NIVA - Norwegian Institute for Water Research / Section for Ecotoxicology and Risk Assessment; J. Heuschele, University of Oslo / Section for Aquatic Biology and Toxicology

Zooplankton shows strong behavioural responses to both biotic and abiotic stressors, e.g., avoiding harmful ultraviolet radiation (UVR) by retreating to deeper waters. Anthropogenic pollutants can also influence the swimming behaviour of zooplankton, e.g., *via* info chemical effects. Yet, an effective experimental approach to measure sensible behavioural endpoints remains a challenge. As behaviour is typically extracted from video recordings, the resulting data analysis is not a simple spreadsheet job either. In this study, we provide a framework for analysing and quantifying swimming behaviour of zooplankton, deriving multiple behavioural proxies from the same experiments. As a case study, we use the swimming response of *Daphnia magna* to UVR, both in the absence and presence of a dissolved organic matter (DOM) gradient. DOM causes a characteristic browning of the watercolour with increasing the light absorption of water, especially in the UVR spectrum. Making use of a custom-built, Raspberry Pi-based imaging system, we repeatedly filmed individual *Daphnia magna* neonates over six hours. We examined the video recordings using the OpenCV library to extract individual position data, and calculated motility proxies such as explored area, swimming speed, swimming depth, travelled distance, and swimming time. These were analysed in R using linear mixed-effect models. To account for the complexity and variability of swimming behaviour, not only UVR and DOM were used as explanatory variables, but also the size of the neonates and the time into the experiment, and all possible parameter interactions were included. The results show that increasing DOM generally buffers the strong effects of UVR on swimming behaviour while raising the overall swimming activity independent of UVR exposure. Our findings highlight the complexity of behavioural responses, which traditional ecotoxicological data analysis approaches might miss. We further emphasise the importance of an in-depth data analysis, both to avoid false results, but also to account for the effect sizes of additional factors like body size and experimental duration on behavioural responses. Wolf R, Heuschele J. 2018. Water browning influences the behavioural effects of ultraviolet radiation on zooplankton. Front Ecol Evol 6:26. doi: 10.3389/fevo.2018.00026.

WE270

Effects of Per- and polyfluoroalkyl substances (PFASs) on zebrafish embryo behaviour

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Per- and polyfluoroalkyl substances (PFASs), a group of omni-present and very persistent environmental pollutants, cause concerns regarding their environmental impact and their risk for human health. The zebrafish (*Danio rerio*) embryo is a robust model organism for the evaluation of the developmental toxicity of chemicals, but also more sensitive endpoints, e.g. behavioural endpoints, have become more popular in recent years. In the present study, zebrafish embryos were used to investigate behaviour alternations caused by PFASs. Special focus was put on the comparison between single substance and mixture effects. First, based on control data, a set of locomotor activity endpoints was defined to establish a normal swimming profile of 6-day-old zebrafish larvae. Then, the behaviour of exposed groups was investigated. In total, nine PFASs plus a PFAS mixture were investigated at seven concentrations ranging from environmentally relevant to acutely toxic levels of certain PFASs. Significant differences were detected in the swimming distance, the movement composition and the reaction to light change of the exposed larvae. In general, toxicity increased with increasing length of the perfluorocarbon-chain and the active group of the PFASs seemed to influence the toxic response. PFAS mixture toxicity generally followed the trends of single substances, but in cases where different toxic responses were observed, mixture toxicity could not be predicted. More complex models are needed to predict the mixture toxicity of PFASs as a simple additive toxicity model is not sufficiently.

WE271

Effect of climate change temperatures on the toxicity of the pesticide fipronil
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There is a broad consensus in the scientific literature that the most serious effects of global warming are related to a change in average global temperatures. These changes include an increase in the surface water temperature of lakes and streams, especially at high altitudes that occur even faster than the warming of the ocean or atmosphere. Such an increase could have dramatic effects on aquatic ecosystems in the medium and long term. Additionally, pollution as a consequence of human activities, exposes aquatic communities to a scenario of multiple stress. Therefore, there is a growing concern to know how contaminants present in fresh waters will affect these ecosystems in future complex exposure scenarios Fipronil is a phenylpyrazole insecticide widely used as a phytosanitary product and veterinary medicine, due to its great effectivity against a broad spectrum of insects. This insecticide acts by targeting the receptor for c-aminobutyric acid (GABA) and has a higher binding affinity to GABA insect receptors than to mammals. Our study evaluates the impact of fipronil, together with the temperature, on *Daphnia Magna*, an aquatic invertebrate selected as biomarker for freshwater, and also in a fluvial microbial community. We tested the effect of fipronil at three temperatures (18,23 and 28 ^{0}C) on the acute ecotoxicity on *D. magna* and also on the growth and physiological diversity of the river bacterial communities. Results indicate that the toxicity of fipronil is higher the higher the temperature. Fipronil affects both the survival of *D. magna* and the ability of the river bacterial community to metabolize substrates. These results suggest that the toxicity of some pesticides in freshwater ecosystems can be enhanced with the increase of temperatures in the expected range due to climate change. The authors thank the financial support of MINECO-FEDER (CTQ2015-64049-C3-2-R), Gobierno de Aragón-FSE (GATHERS E39_17R) and Cátedra Novaltia.

WE272
Does low-level radioactive contamination cause developmental instability in chironomids?
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There has recently been increasing interest in protecting non-human wildlife from the effects of ionizing radiation. However, only limited data are available on radiation effects on wildlife species and ecosystems, and methods for detecting such effects are not well developed. In this study, we investigated the effects of low-level radioactive contamination on chironomid midges. Developmental effects were evaluated by observing time of emergence of adult insects after exposure of larvae, and by measuring fluctuating asymmetry (FA) in the wings of the adults. FA has been suggested to be a sensitive measure of developmental instability induced by environmental exposures. Larvae of *Chironomus riparius* were grown in controlled laboratory conditions in sediments from two contaminated ponds (higher and lower contamination level) located near an abandoned uranium mine and a reference pond. Artificial sandy sediment was used as an additional control. Wing size measurements were used for assessment of FA. Fish food (TetraMin®) was added at two feeding regimes (12 and 36 μl/beaker/day). Emergence of the adult midges was significantly delayed by both contaminated sediments. However, FA was affected only by the higher contamination level, and only at the lower feeding level. In conclusion, it appears that FA evaluated by wing size measurements is not a more sensitive indicator of developmental effects than time to emergence. **Key words:** Fluctuating asymmetry; Chironomids; Wing measurement; Radioactive contamination

Disinfection Byproducts and Oxidation Byproducts: Analysis, Fate, Toxicity and Treatment (P)

WE273
Leucomethylene blue, a selective photometric reagent for chlorine dioxide analysis in water. Part II: the sulphate interference
R. Devesa, Aigues de Barcelona / Chemistry. Laboratory; N. Jiménez, Polytechnic University of Catalonia, Barcelona Tech / Chemical Engineering; A. Garbayo, Agbar, Barcelona Water Company / Analytical Chemistry; F. Estrany, Polytechnic University of Catalonia, Barcelona Tech
Drinking water supply companies have traditionally focused their efforts on providing a product with health guarantees, a safe and clean water. Disinfection has been the main purpose of the water treatment. A broad range of disinfectants and technologies are available. Chlorine dioxide has been increasingly used because of its ability to avoid the formation of trihalomethans (THMs), the most common and well-known disinfection by-products. N,N-Diethyl-p-phenylenediamine (DPD) method has been without any doubt the most common procedure for the analysis of free and combined chlorine (chloramines) and also, chlorine dioxide. While this method easily permits the differentiation between free and combined chlorine, the selectivity of the analysis of chlorine dioxide when chlorine is present has recently been questioned. As a result, this procedure has been removed from Standard Methods (American Water Works Association) and qualified as "reserved" method. Given this circumstance and the need of having a selective method for chlorine dioxide, several UV-VIS spectrophotometric methods have been evaluated by our group. In a previous study (1) leucomethylene blue, a chromophore agent obtained by reduction of methylene blue and scarcely described in the literature for chlorine dioxide analysis, showed good analytical performance. But the measurements were interfered by high concentrations of sulphates. In this study, two alternatives to solve the interference have been evaluated: microfiltration after the precipitation of sulphate with barium chloride and solvent extraction with 1,2-dichloroethane. The filtration method gave better results: first, the measurements presented a good reproducibility; secondly, there was not significant interference by free chlorine, which is frankly important for the water supply monitoring; and third, the method showed a good accuracy with real water samples (relative error below 20 % for chlorine dioxide concentrations between 0 and 1.6 mg/L.). This reagent with this method has revealed to be the best option among the different compounds that we have tested – amaranth, lissamine green, and choro phenol methyl red- in previous studies. (1) Leucomethylene blue: a selective photometric reagent for chlorine dioxide analysis in water. R. Devesa, X. Aldazabal, A. Garbayo, F .Estrany. 28th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC). Rome, Italy, May 2018

WE274
Water treatment - A regulatory challenge for crop protection products in the EU
S. Dorn, F. Schnitzler, knoell Germany GmbH
Regulation (EC) No 1107/2009 lays down the rules for the authorisation of crop protection products in Europe with the aim of protecting humans and the environment. It is known that during primary disinfection processes for central water treatment (e.g. ozonation, chlorination) certain active substances included in crop protection products and their metabolites have the potential to form unwanted by-products with e.g. toxic, carcinogenic and genotoxic characteristics. Notwithstanding that the EFSA (European Food Safety Authority), the scientific advisory body of the European Commission, has recently identified data gaps during the approval process of active substances, water treatment processes have not (yet) been implemented in the European data requirements (Reg. 283/2013 or 284/2013) relevant for the authorisation of crop protection products. Further, no guidance document for experimental testing is available. With this information pending, addressing water treatment processes successfully becomes a challenge for applicants of crop protection products. The European Commission states that the applicant shall submit the (confirmatory) information to the European Commission, the Member States and EFSA within two years after adoption of a guidance document on evaluation of the effect of water treatment processes on the nature of residues present in surface- and groundwater. Meanwhile applicants seek to address the issue in their dossiers to authorities in different ways – from simple waiving to the generation of extensive supporting data. Current attempts of addressing ozonation and chlorination for active substances from the third Annex I Renewal programme (AIR 3) are summarized based on publically available data. Following most recent developments in the area, the poster will also review the current activities and issues around the implementation of the upcoming guidance document: Currently water treatment is only addressed at active substance level. It remains largely unclear how the topic can be approached for product registrations and how metabolites can be realistically addressed. With such guidance in place it can be expected that other chemical sectors, e.g. industrial chemicals or pharmaceuticals, would also need to set a spotlight on the topic.

WE275
ECOTOXICOLOGICAL COMPARATIVE EVALUATION BETWEEN CHLORINE AND OZONE DISINFECTION OF MUNICIPAL WASTE WATER PRODUCED IN A CONVENTIONAL ACTIVATED SLUDGE TREATMENT PLANT IN BRAZIL
C.H. Soares, Universidade Federal de Santa Catarina / Biochemistry Department; G. Costa, UFSC; I. Baptista, Universidade Federal de Santa Catarina
The conventional sewage treatment plants need a disinfection process step to comply with the legal standards to dispose the treated waste water on the environment. The chlorine is the most economically attractive option. However, it has a residual effect and potential carcinogenic by-products formation. The ozone is more expensive, but due to its high reactivity it does not present residual effect. (ZIETZSCHMANN,2015), nevertheless it has a known existence of toxic by-product formation (RICHARDSON et al, 2007). The ecotoxicological evaluation is more sensitive and representative of the impact of an effluent the environment. With the aim of assess and measure the ecotoxicology potential, took place the waste water sampling in a treatment plant constituted by an UASB reactor, activated sludge reactor and disinfection via gaseous chlorine. The samples consisted in: raw effluent, decanted, chlorinated and ozonated. In laboratory scale a pilot ozonization reactor was set up to treat the decanted sampled waste water to compare with the chlorine system. To better explanation of the fact, some physical chemical parameters ware measured: total phenol concentration, pH, Conductivity, COD, DBO, Ammonia nitrogen, nitrate, total phosphate, sulfide. The ecotoxicological analysis were made with the following tests and

bioindicators: Acute and chronic test using the micro crustacean *Daphnia magna*, and exposition of *Danio rerio*'s embryos and following's development (the aim was to evaluate the sample's teratogenicity with a higher sensibility). The ozonated samples had better performance as well as on physic-chemical parameters as the phenols, color and organic matter degradation and on ecotoxicological tests, being one of the reasons for lower ecotoxicity probably was the ammoniacal nitrogen (more toxic) conversion to nitrate, less toxic form of nitrogen, promoted by ozone treatment.

WE276

Mapping degradation pathways in advanced oxidation processes with two-dimensional liquid chromatography with an online light-degradation system

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While advanced oxidation is applied in drinking water treatment, recent studies [REF] indicate that after medium-pressure UV/H_2O_2 treatment mutagenic substance are formed. Due to the complexity of water samples the origin of these mutagenic components is unclear. This problem can be split in two. First, one chromatographic dimension is not able to separate the complex water sample. Secondly, there is no knowledge the photodegradation pathways. This projects aims to solve both of these problems.\n This poster will introduce the TooCOLD project (Toolbox for studying the Chemistry Of Light-induced Degradation), conducted within several institutes at the University of Amsterdam (UvA) and the Free University Amsterdam (VUA). In this project, the complexity of the water samples including the Natural Organic Matter (NOM) will be analysed with two-dimensional liquid chromatography (2DLC) in combination with mass spectrometry. Next to applying 2DLC-HRMS to water-treatment samples, the samples will be subjected to an online-degradation system. After the first-dimension separation, the components will separately be trapped in a photo-degradation cell. In this cell the analytes are continuously illuminated at any desired wavelength(range). In a second separation dimension after the exposure cell the degradation products will be separated and analysed with mass spectrometry and UV. In this way we can establish a link between the degradation products and the starting materials. \n Apart from water research, this 'Toolbox for Studying the Chemistry Of Light-induced Degradation' (TooCOLD) will be applied on samples from cultural heritage and the food industry.

Individual Variation in Ecotoxicological Research: a Change from Unwanted Noise to a Meaningful Endpoint (P)

WE277

Genotype variation in heavy metal tolerance can drive inter- and intra-specific selection amongst Baltic Sea diatoms

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The Baltic Sea is one of the most polluted seas in the world. Microalgae are key primary producers in the Baltic Sea, as in other aquatic environments. These organisms have short generation times, flexibility in reproductive strategies, and often high standing genetic variation, which should facilitate rapid evolution. We explored whether evolution and inter-specific competition can lead to modifications in heavy metal tolerance in Baltic Sea microalgae. We used the sediment seedbank to revive individuals from two ecologically important pelagic diatom species, *Skeletonema marinoi*and *Thalassiosira baltica,* at a mining polluted inlet of the Baltic Sea (Gåsfjärden). We investigated if long-term exposure to mining discard has resulted in more heavy metal tolerant diatom individuals, both within and between the two species. We found that, generally, *T. baltica* was more tolerant to silver (Ag) and copper (Cu), whereas *S. marinoi* was more tolerant to cadmium (Cd) and lead (Pb). This suggests that mining discharge can have different effects on the structure of phytoplankton assemblages, depending on the composition of metals. In 72h acute exposure tests we also observed an elevated tolerance to Cd and Cu within the *S. marinoi*population at Gåsfjärden suggesting that the species has evolved heavy metal tolerance at this location. We are currently investigating the mechanism and scale of this evolution using long-term (42 days) artificial selection experiments on assembled *S. marinoi* populations. We use population genomics and physiological measurements of metal-tolerance to assess differences between genetic individuals. Our results add valuable scientific information about the population-wide variance in heavy-metal sensitivity of marine diatoms as well as the evolutionary capacity of this group of globally important primary producers.

WE278

Crude oil has transgenerational effects on phenotypic plasticity and individual variation of metabolic rate of Daphnia magna

K. Anttila, E. Suominen, University of Turku / Biology; M.J. Nikinmaa, University of Turku / Department of Biology

Worldwide animals are under threat of crude oil spills and especially in Baltic Sea the threat is serious. This is because 11% of the global shipping of crude oil happens through Baltic Sea which waterways are shallow and narrow. Although there are several studies of physiological effects of crude oil and its water soluble fractions (WSF) on different organisms, little is known about its long lasting transgenerational effects. In this study we exposed offspring of single *Daphnia magna* to 30%, 10% and 0% (control) WSF for 48h and measured its effect on oxygen consumption rates of animals. The highest exposure caused 50% mortality of the animals and this led to significant reduction in individual variability inside population as compared to other exposures (p=0.008). There were no significant differences in the mean oxygen consumption rates between animals from different exposures (p>0.05). However, the reduction of individual variability in 30% WSF exposure was reflected in the oxygen consumption rates in the next two generations. The offspring of animals exposed to 30% WSF had significantly higher oxygen consumption rates than the offspring from other groups (p< 0.001 in both F1 and F2 generation), which showed reduced oxygen consumption rates as compared to parental individuals (p=0.003 in control animals and p=0.004 in 10% WSF-exposed animals). As conclusion, the reduction in individual variability after WSF exposure could make the animals vulnerable to other environmental hazards since the buffer capacity in the population lowers. The exposure also had transgenerational effect by elevating oxygen consumption rate, i.e. animals are spending more energy for the same activities. This could have long lasting negative effect on the fitness of the whole population especially if the variability between individuals is reduced.

WE279

Transgenerational effects of crude oil and heat shock on the mortality and oxygen consumption rate of Daphnia magna

J. Eklund, M.J. Nikinmaa, University of Turku / Department of Biology; K. Anttila, University of Turku / Biology

Baltic Sea is a compact area with heavy shipping activity, and since around 160 million metric tons of oil are transported annually, the risk of a major oil tanker spill is ever-present. Since climate change will raise sea temperatures forcing organisms to adapt or die, it is important to determine how the combined stress of oil exposure and rising temperature affects organisms. We studied *Daphnia magna*, since with its parthenogenetic reproduction and short generation time, we could easily follow the effects of exposures on the next generation, after the oil exposure is over. Our hypothesis is that the combined oil exposure and heat shock will lower the viability of *D. magna* and the effects are transferred to next generation. The first generation (F0) of the fleas was exposed to oil water soluble fraction (WSF) concentrations of 0 % (control), 10 % and 30 % for 24 hours, and the day after that to 30 °C heat shock for 24 hours (with 20 °C as control). We measured the mortality and oxygen consumption rate of *D. magna* after both exposures. We left the first generation to reproduce, measuring the number of offspring (F1-generation) and their oxygen consumption rate, while continuing to monitor the F0 mortality. Both 30% WSF exposure and heat shock alone caused mortalities in F0-generation, but the mortality after heat shock was more than doubled in the population that received both treatments, when compared to population that experienced only WSF 30 % exposure (40 % vs. 13 %, respectively). Exposure to WSF 30 % and heat shock caused a significant decrease in oxygen consumption rate in F0-population (p< 0.001). We also found that the WSF and heat shock exposures had significant interacting effects on the oxygen consumption rates of the next generation. For the WSF 30 % + No-heat shock –F1 population, oxygen consumption rate was increased (p=< 0.001) compared to WSF-control + No-heat shock, but no similar effect was seen in WSF 10 % + No-heat shock. In the heat-shock F1 population, WSF caused a decrease in oxygen consumption rate compared to WSF-control + heat shock (p=< 0.001 in both WSF concentrations) along with a decrease in individual variation. We conclude that the lowered oxygen consumption rate in F1 generations of parents that are exposed to WSF and heat shock is a response to stress, which together with the reduced variation could have negative effects on populations exposed to both oil and increased temperatures.

WE280

Phenotypic plasticity of thermal and copper tolerance in three-spined sticklebacks (Gasterosteus aculeatus) from natural and thermally polluted areas.

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Climate change will increase the frequency and duration of extreme thermal events as has been seen during summer 2018 in the whole northern hemisphere. These heat waves have led to mass fish death events around the world. Moreover, the presence of a certain pollutant might further compromise the ability of ectotherms to respond to these sudden temperature changes. The capacity of fish to cope with an anthropized environment can be exerted throughout a mechanism of phenotypic plasticity. There are, however, only few studies how fish that have experienced long term (decades) increase of temperature in their habitat are able to respond to both heat waves and presence of pollutants, such as heavy metals.

The capacity of individuals to change their tolerance will, nevertheless, define survival capability of entire populations. Here, we evaluate the thermal plasticity of critical thermal maximum (CTmax) in six populations of three-spined sticklebacks exposed to copper (100 μg/L) for one week, in laboratory conditions. Populations were from coastline and from areas that have been warmed by ~10°C by nuclear power plants (NPP) for four decades in Finland. According to results the decades of warming has not increased the thermal tolerance of sticklebacks. Surprisingly, all the individuals from each population were able to increase their tolerance after copper exposure by ~1°C ($p < 0{,}001$). Further molecular studies will be conducted about the molecular response of Heat Shock Proteins. We propose that decades of warming has not reduced the capacity of three-spined sticklebacks to respond to environmental contamination. Yet, they possess some phenotypic plasticity to respond to copper exposures.

WE281

Characterising within-species individuality in the escape response of a marine amphipod

S.A. Kohler, University of Portsmouth / Aquatic Biology; A. Ford, University of Portsmouth / Biological Sciences; M.O. Parker, University of Portsmouth

Animal behaviours are increasingly recognised as a useful endpoint in ecotoxicological studies, as they can provide a link between the ecological and biochemical effects of compounds. However, within-species variation can impact the results of behavioural assays. Studies on fish have shown that classification into bold and timid individuals prior to exposure, allowed for more accurate interpretation on the effects of exposure to behavioural modifying compounds. Currently, there is paucity of data on baseline unconditioned behaviours in aquatic invertebrates. Previous studies in our labs have shown behavioural data to be highly variable and potentially complicated by behavioural phenotypes. We have shown that the marine amphipod *Echinogammarus marinus* is negatively phototactic, however when stimulated by light, individual responses can vary from 'responders' to 'non-responders'. In this study, the baseline unconditioned response of 70 *E. marinus* to sudden exposure to light was measured in a DanioVision observation chamber with EthoVision tracking software. The software recorded data every 0.033 seconds and mean velocity was used as a measure of swimming activity, the initial 2 seconds of response to sudden light stimulus was measured providing 60 data points per organism. Individuals of *E. marinus* exhibited different responses to light, with organisms showing either an increase or decrease in swimming activity. *E. marinus* are an intertidal species which are predated upon by different organisms at low or high tide. This difference in response may be an evolutionary adaptation to dealing with mixed predators which hunt using different sensor cues. Our work suggests that pre-organising your organisms into positive or negative response groups may reduce noise in behavioural data for ecotoxicological studies. The role of phenotypic plasticity and interpretation of behaviours in ecotoxicology are discussed

WE282

Analysis of inter-individual variability in locomotor activity of larval zebrafish as a basis to improve neurotoxicity testing of chemicals

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Many environmental contaminants, such as pharmaceuticals, pesticides and heavy metals, have been shown to interfere with the nervous system of different species including humans and fish, thereby creating deficiencies in sensation and behavior. Behavior is the integrated output of multisensory, neuroendocrine and neuromuscular signals and is an important, ecologically relevant and very sensitive endpoint of toxicity measurement. In aquatic toxicology, the analysis of locomotor behavior of chemically exposed zebrafish larvae has become a prominent method for the assessment of neurotoxicity, because of the easy-to-handle, high-throughput and automated manner of testing. However, the detection of group differences can be hampered by high inter-individual variability among larval zebrafish. Therefore, the aim of this study was to investigate behavioral inter- and intra-individual variability in zebrafish larvae as a basis to better estimate effects of chemicals on behavioral responses. We assessed the variability of locomotor behavior in larval zebrafish from 5 to 7 days post fertilization for some frequently used protocols (spontaneous swimming, light/dark phases, tapping stimuli) and collected physiological and morphological data of the individual larvae in order to explore whether they correlate with locomotor activity. Our results show that the variability in locomotor activity is lowest during dark phases, but for morphological parameters (length of the larva, size of the swim bladder, size of the yolk) and cardiophysiological measurements (heart rate), there was no correlation with individual locomotor activity. When looking into consistency of activity of the individuals, we found that during dark phases, activity levels correlated well between measurements taken in the morning and afternoon of each day and the activity of individual larvae was highly consistent between day 6 and 7. Overall, our study indicates that taking the variability of locomotor activity into account will improve the evaluation of chemical effects on behavioral responses of larval zebrafish and has the potential to reduce the number of animals to be used for neurotoxicity testing. These results might also offer new insights into mechanisms of toxicity considering that every individual's response to a chemical differs based on their genetic make-up.

WE283

Local genetic adaptation to pesticide use in organic and conventional agriculture

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The impact of organic farming is expected to differ from that of conventional farming. Organic farming has therefore been highly promoted under the current EU Common Agricultural Policy. To date, most comparative studies on the impact of organic and conventional agriculture focus on the agricultural land itself, on terrestrial ecosystems, or on rivers. Yet, small farmland ponds harbor much of the regional aquatic biodiversity and are key providers of vital ecosystem services. We will present the first results from a common garden experiment testing to what extent organic and conventional agriculture differentially affect local genetic adaptation of a non-target, sentinel species, the water flea *Daphnia magna*. *Daphnia* are key grazers in lentic waterbodies, and thus contribute strongly to the ecological integrity and ecosystem services of farmland ponds. Earlier investigation demonstrates that *Daphnia* populations can genetically adapt to pesticide use in agricultural areas. We collected multiple clones from different populations (n=7) of *Daphnia magna* from ponds located in conventional agriculture, organic agriculture and natures reserves. The collected clones were cultured under laboratory conditions for at least two generations to minimize interference from maternal effects. A common garden experiment was used to quantify the sensitivity of *D. magna* populations to two pesticides typically used in conventional (Chlorpyrifos and Imidacloprid) and two pesticides used in organic agriculture (Pyrethrine and Copper Sulfate). The EC_{50} value for the different populations and pesticides was determined by exposing juveniles (< 24h old) to eight different concentrations of each pesticide for 48h. The results of this study will provide important insight on the genetic consequences of agriculture on non-target species and can be highly relevant for policies focusing on the development of more sustainable agricultural schemes.

WE284

Effect of test medium components on metals toxicity in microalgae

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Metals happen naturally in the environment, however, anthropogenic activities highly contribute to increasing their concentration in aquatic ecosystems, becoming water pollution one of the biggest concerns around the world. The toxicity assessments following standardized guidelines are indispensable for water quality regulation and environmental risk assessment. However, reported values of critical concentration of toxicity such as the median effective concentration (EC50) are inconsistent, even the same testing species were used. One of the reasons for this wide variation in reported in previous studies can be attributed to the conditions of test media. Media with high nutrients concentrations are commonly used in assessments using microalgae, but such nutrients amounts are not observed in natural environments. Moreover, the chemical interaction of nutrients and tested metal may also affect the outputs of toxicity assessment. The purpose of this research was to evaluate the effect of copper (Cu), lead (Pb) and zinc (Zn) on microalgae *Pseudokirchneriella subcapitata*, using three different types of culture media, including Bold Basal Medium (BBM), OECD medium and AAP medium. The assessment was carried according to the OECD guideline. Algae were exposed to the metals for three days using a borosilicate flask, and pH was adjusted to 6.5. The fluorescence intensity at 435/685 nm of excitation/emission wavelength was measured every day to follow the algae growth. The specific growth rate was estimated using the formula provided in the OECD guideline and EC50 estimated using the log-logistic dose-response curve. The estimated EC50 values using OECD, BBM and AAP media were 38.5 μg/L, >3000 μg/L and 95.5 μg/L, respectively for Cu, >3000 μg/L, >3000 μg/L and 117.8 μg/L, respectively for Pb, and 155.4 μg/L, >3000 μg/L and 146.2 μg/L for Zn. These results indicate that Pb is apparently less toxic than Cu and Zn. The BBM medium gave extremely high EC50 (>3000 μg/L) for all tested metal species, which also allowed higher growth rate of the microalgae because of the highest nutrient concentrations among the used test media. The EC50 value of Cu was higher in the OECD medium than AAP medium, but the opposite result was obtained for Zn. This means that components of the test media influence to the metals toxicity. Further research should be done to determine the real metal toxicity on the microalgae growth, considering the effect of the chemicals in the test media.

Contaminated Sediments: an Understudied Environmental Compartment (P)

TH001

Persistent Organic Pollutants in sediments from rivers in Moldova

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and Ecotoxicology; L. Ahrens, Swedish University of Agricultural Sciences SLU / Dept. of Aquatic Sciences and Assessment; K. Wiberg, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; A. Dahlberg, Swedish University of Agricultural Science / Department of Aquatic Sciences and Assessment

Being predominantly an agricultural country, Republic of Moldova has extensively used organochlorine pesticides in the past, compounds which nowadays are banned and listed as persistent organic pollutants (POPs) under the Stockholm Convention. There are limited data in the open literature on POPs in aquatic ecosystems from Moldova. Sediments act as a sink for various chemicals but can also serve as a source for dispersal of POPs to the surrounding ecosystems. In this study, hexachlorobenzene (HCB), 20 polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its transformation products (DDE and DDD) were analysed in sediments collected from the two main rivers in Moldova (Dniester and Prut River). The sediments were sampled at a depth of 0-5 cm at 14 different sites during the summer of 2017. The sediment samples (*n*=14), which consisted of three pooled subsamples (per sampling site), were Soxhlet extracted, cleaned-up using several steps, and finally analysed using gas chromatography coupled to mass-spectrometry (GC-MS/MS).The concentrations profiles were generally $?_6$DDTs ≥ $?_{20}$PCBs ≥ HCB in both rivers. The average concentration of HCB was 0.2 ng g^{-1} dw in the Dniester River and 0.080 ng g^{-1} dw in the Prut River. The highest concentration of HCB was found at Soroca sampling site (0.98 ng g^{-1} dw, Dniester River) and at Giurgiulesti sampling site (0.14 ng g^{-1} dw, Prut River). The average concentration of $?_{20}$PCBs was 2.4 ng g^{-1} dw in the Dniester River and 0.74 ng g^{-1} dw in the Prut River, with the highest concentration at Goieni sampling site (9.2 ng g^{-1} dw, Dniester River) and Leuseni (1.5 ng g^{-1} dw, Prut River). DDT and its transformation products were found in all samples. The concentrations of $?_6$DDTs ranged from 1.1 to 52 ng g^{-1} dw in the Dniester River and 3.5 to 89 ng g^{-1} dw in the Prut River, with maximum values at Goieni and Leuseni. *p,p'*-DDE and *p,p'*-DDD were the predominant compounds and constituted 56% and 26% of $?_6$DDTs in the Dniester River and 47% and 33% in the Prut River, respectively. The predominance of transformation product reflects historical use of DDT and indicates significant agricultural input in the past in Moldova. Five sites from the Dniester River showed levels of $?_4$DDT that could cause chronic effect on sediment dwelling organism at long term exposure, according to Norwegian environmental quality standards. Our study suggests that further monitoring of POPs in aquatic ecosystems in Moldova is needed. \n \n

TH002

Application of artificial neural network (ANN)-self-organizing map for the categorization of sediment contaminated by PAHs in Serbian's stretch of Danube River

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The Kohonen self-organizing map (SOM) is a type of Artificial neural networks (ANNs) that uses unsupervised learning for data training to classify and adequately recognize patterns embedded in the input data space. In this study, SOM–ANN was used for recognizing spatial patterns in polluted areas by unificating physico-chemical, ecotoxicological and toxicokinetic variables in the identification of contamination sources and similarities in the quality of the sediment samples. Ten Danube sediment samples contaminated with 16 priority polycyclic aromatic hydrocarbons (PAHs) were quantified by gas chromatography–mass spectrometry. The total concentration of 16 PAHs ranged from 99.48 to 520.48 ng/g, with a mean (median) concentration of 271.86 ng/g (235.37 ng/g). Individual PAH analysis showed that PAHs with four to six rings were the most frequently detected isomers and accounted 85% of the total PAHs concentrations. Correlations and relationships between the samples and the variables were determined by comparing the component planes with similar patterns of the self-organizing map (Fig 1). The SOM network was trained using a 7 × 7 map. The results have highlighted the dependencies between the different PAHs and the classification of studied sediments into four classes with similar pollution levels. The first (Nap, Acy, Ace, Fl) and second pattern (Phe and Ant) were dominated by LMW PAH contaminants, which are likely released from similar petrogenic sources. The third pattern including Flu, Pyr, B(a)A, and Chr, which are HMW PAHs commonly discharged from coal combustion. The final pattern comprises B(b)F, B(k)F, IP, and B(g,h,i)P, which are also HMW PAHs from pyrogenic sources. Principal component analysis (PCA) and hierarchical classification of SOM maps (SOM AHC) were also used for validating the obtained results. The proposed methodologies and results obtained in this paper provided valuable assessment using the SOM visualization capabilities and highlighted zones of priority that might require additional investigations and also provide productive pathway for effective decision making and remedial actions.

TH003

Polychlorinated biphenyls and naphthalenes in sediments of a contaminated river in Finland: Levels, sources and bioavailability

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The pollution of a Finnish river, the River Kymijoki, with polychlorinated biphenyls (PCBs) and naphthalenes (PCNs) was investigated by sediment data. This river descends to the Gulf of Finland and has been a secondary source of pollutants to the Baltic Sea due to contamination via several plants, including pulp mills, a chlor-alkali plant and a former chlorophenol plant. Variable levels were measured for the PCBs (from 12 to 390 µg/kg dry weight, dw) and PCNs (from 0.83 to 13 µg/kg dw) in surface sediments, which had been collected from different locations of the river, the PCBs being more abundant than the PCNs in all sites. The levels of both pollutant groups exceeded those measured in the Finnish coast of the Baltic Sea. Analyses of a core from the vicinity of the chlor-alkali and a former chlorophenol plant revealed that historical burden may constitute a significant source for both the PCBs and PCNs in the river. Different spatial distribution of the PCNs compared to that of the PCBs and differences in congener profiles in surface sediments with those of technical formulations suggested that there have been multiple sources for both pollutants to the River Kymijoki. The chlor-alkali plant was considered one source of the PCBs and PCNs, either via the process or use of technical formulations, while elevated levels of the PCBs in the lower reaches of the river could possibly be explained by other industrial processes or leaks of formulations from electrical equipment used earlier in plants. The comparison of the surface sediment data to the data of mussels incubated in this river pointed to low potential for the accumulation of the PCBs and PCNs from sediment to aquatic biota. The continuous transport of pollutants in sediments downstream and into the Gulf of Finland in the Baltic Sea, however, has to be kept in mind.

TH004

Spatio-temporal exposure of Plant Protection Products in OECD 218/219 sediment test systems - Modelling and measurements

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Sediment toxicity testing is currently revised under the premise to improve quality and consistency of regulatory risk assessments. In 2015, EFSA has published a scientific opinion on risk assessment for sediment organisms where sediment-spiked (OECD 218) and water-spiked test systems (OECD 219) are considered to study chronic effects on sediment organisms. Prominent test organisms are Chironomids, aquatic insects which live in and on soft sediments. Usually specific mean concentrations of the overlying water or over the entire sediment are used to derive effect endpoints. However, due to the design of these studies initially large gradients between the exposure in the overlying water and in the sediment layer are established. As a consequence, substantial temporal and spatial dynamics of local concentrations have to be expected, especially in the vicinity of the interface beween water and sediment where the Chironomids larvae are supposed to live (observations made in several internal studies). Following up previous work (Hammel et al, 2018) we simulated the transport and the redistribution of four active ingredients of plant protection products with the mechanistic model TOXSWA. Studying a number of compounds from high to low mobility (with Koc from zero to several thousand) and spiking either overlying water or sediment allowed a comprehensive analysis of compound- and test-specific local concentrations in such test systems and their temporal behaviour. The results of the simulations are compared with measured sediment concentrations in three depths (see contribution submitted by Dorn et al.). Main findings are that both, compound mobility and mode of spiking have a major impact on local concentrations. Presuming that chironomids live in the upper millimetres of the sediment their exposure can be very different from the average concentration depending on substance mobility and test design. This approach allows to identify the ecotoxicologically relevant exposure which should be considered for the derivation of effect endpoints of chironomids. It also allows to set water- and sediment-spiked studies for a specific substance into context because in both studies the underlying dose-response should be the same. Hammel K., Dorn A., Dalkmann P., Bruns E. and Faber D (2018). Spatio-temporal exposure of Plant Protection Products in OECD 219 sediment test systems - Comparison of model results with measurements. Platform presentation SETAC Europe 2018 conference

TH005

New insights of the relevant ecotoxicological exposure pathway of Chironomus riparius in OECD 218/219 tests

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The European Food Saftey Authority (EFSA) published in 2015 a scientific opinion on environmental risk assessment for sediment organisms. This includes a discussion on how regulatory acceptable concentrations for sediment organisms could be derived and what is the relevant exposure pathway for test organisms such as *Chironomus riparius*. To challenge the assumption that pore water over the entire sediment layer is a relevant route of exposure for *C. riparius*, we conducted over the past two years several studies in-line with OECD 218/219 to investigate the spatio-temporal behaviour of different compounds in the water-sediment system. The compounds differed in their physico-chemical characteristics like the logKow. In the studies, analytical measurements were performed at different times for the overlying water and in 3 sediment core samples divided into 3 x 5 mm-layers each for the total sediment and the respective pore water concentration. This improvement of the sampling allowed us to have a closer look at the local concentrations at different times in the test system. In parallel, the measurements were compared with predictions by TOXSWA model. Furthermore, it was investigated where the *C. riparius* larvae live in this system prepared with artificial sediment. The approach and first results were already presented at the SETAC in Brussels 2017 (Herno, V. et al.) and in Rome 2018 (Hammel, K. et al.). The new results show, that *C. riparius* larvae live in the upper first mm of the sediment and are therefore predominantely exposed to a concentration comparable to the overlying water instead of the average pore water concentrations of the sediment layer. The analytically measured and predicted TOXSWA concentrations show that the compound mobility and the mode of spiking have a major impact on the local concentrations in the system (see contribution submitted by Dorn et al. & Hammel et al). Taking this into consideration it is important to combine the living behaviour of the test organism with the local concentration of the compound to predict the relevant ecotoxicological exposure pathway and to base further regulatory decisions on these circumstances. Herno, V. et al., (2017): Pore water – The relevant exposure pathway for sediment risk assessment? Setac Europe 2017 Hammel, K. et al., (2018): Spatio-temporal exposure of Plant Protection Products in OECD 219 sediment test systems - Comparison of model results with measurements. SETAC Europe 2018

TH006

Effects of bioturbation on the freely dissolved hydrophobic organic chemical contribution in sediment cores of the German Bight

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Marine muddy sediments store a large amount of legacy hydrophobic organic chemicals (HOCs), such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). The primary emissions to the environment of many HOCs have been reduced as a result of strict regulations. Undisturbed sediment serve as sink for HOCs and exhibit a normal distributed chemical profile according to the historical input of HOCs in the environment. For example, the PCB concentration first increases with depths until a maximum is reached and then decreases again with depths according to the temporal industrial use. The release of HOCs from the sediments to water and biota pose a risk of negative effects on the local ecosystem. Especially the benthic community is directly exposed to organic pollutants in the sediment. However, the reworking activities of benthic organisms, a process called bioturbation, can enhance the sediment-to-water flux of HOCs. The organisms' activities disturb the sediment profile and mobilize the chemicals from deeper layers. Beside the chemical analysis of the total sediment concentration (C_{total}), the determination of the freely dissolved pore water concentration (C_{free}) is more crucial since it accurately reflect the bioavailable fraction. Based on C_{free}, the chemical activity (a) can be determined, which describes the potential for ecologically relevant spontaneous processes such as diffusion, bioconcentration and baseline toxicity. In this study, C_{total} and C_{free} of sediment cores from the German Bight (North Sea) were investigated. Recent advances in equilibrium passive sampling methods (EPSMs) offer a promising alternative to the measurement of C_{total} and support improved risk-based decision making since bioavailability of sediment contaminants can be directly quantified via C_{free}. Currently, freely dissolved concentration will establish as an important endpoint for sediment quality and risk assessment (Mayer et al. 2014). The determination of C_{free} of PAHs and PCBs in sediment pore water was performed using solid phase microextraction (SPME) according to Witt et al. (2009). Furthermore, the community bioturbation potential (BPc) was determined according to Queirós et al. (2013) after benthos analysis. Finally, C_{free} and C_{total} were correlated with the bioturbation potential to identify whether there is a mobilization and redistribution of HOCs in the sediment cores caused by bioturbation.

TH007

The Effect of Organic Carbon on the Availability of Cobalt in Sediments

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Cobalt is produced as a waste product in a number of industries and therefore has the potential to leak into the environment. It is possible it would then contaminate the environment depending on the conditions of the system, such as pH and relative content of cation sorbing phases. We investigated Co sorption to the common sediment and soil constituents ferrihydrite, kaolinite, humic acid, ferrihydrite-HA and kaolinite-HA organo-mineral composites, and three natural sediments with a range of organic carbon contents. Sorption batch experiments, synchrotron X-ray absorption spectroscopy (EXAFS), and thermodynamic surface complexation modelling were used to develop our understanding of the system and determine whether Co behavior in complex systems could be modelled assuming linear additivity of Co sorption to the end-member phases. Sorption to each of the sorbents increased with pH. Sorption to the organo-mineral composites was found to be intermediate between the end-member phases, displaying enhanced sorption respective to the mineral phase at low-mid pH. Sorption to the natural sediments increased with increasing organic carbon. EXAFS analysis shows that at mid-high pH Co sorbs to each of the end-member phases and the organo-mineral composites via inner-sphere bidentate binuclear surface complexes. Our molecularly constrained thermodynamic surface complexation models for Co sorption to ferrihydrite, kaolinite, HA, ferrihydrite-HA, kaolinite-HA, and three natural sediments show that Co sorption to complex systems cannot be modelled assuming linear additivity of Co sorption to the end-member phases.

TH008

SEDIMENT RESUSPENSION EVENTS - THE IMPACT OF CLIMATE CHANGE ON SEDIMENT-BOUND METALS: Geochemistry

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Sediments play an important role as sinks for sequestering contaminants from local ecosystems. However, under future climate change scenarios, more frequent and intense precipitation events may lead to remobilization of sediments and an increase in the flux of contaminants to the local ecosystem. Sudbury, Ontario has been exposed to decades of smelter emissions that have released metal(loid)-bearing particulate matter, which has accumulated in lake bottom sediments. The present study aimed to investigate the influence of resuspension on the fate and effect of metals in Sudbury area lake sediments. This poster outlines the approach and results of investigations into physical-geochemical factors that could influence metal dissociation from particulate matter. To assess mobility of metals, core resuspensions and shaker flask tests were conducted. Results indicated that sediment disturbances, such as would occur during intense storm events, produce a range of geochemical changes and element behavior that differ from control samples that experienced no resuspension. In addition mineralogy and sequential extraction data provided explanations for these geochemical changes, highlighting the importance of a weight of evidence approach to understanding metal fate in a changing climate.

TH009

Assessing bioavailability and toxicity of dual metal-contaminated sediments to early life stages of medaka (Oryzias latipes) fish

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In the river system, sediment is considered as a long-term source of contaminants for aquatic organisms due to its high accumulating capacity. Among all contaminants, heavy metals are of high concern because of their non-biodegradable nature. The bioavailability of these metals may be affected by sediment properties, transportation behavior between sediment-water system and physiology of aquatic organisms. To understand the chemical and biological interaction of sediment-bound contaminants to the aquatic life, this study used the whole-sediment exposure method with intermittent water renewal system to evaluate the bioavailability and toxicity of two heavy metals [i.e. lead (Pb) and copper (Cu)]. The early life stage (ELS) fish of medaka (*Oryzias latipes*) embryos and larvae were exposed to a variety of Pb- and Cu-spiked sediments with different physicochemical properties. The acute mortality and sub-lethal (e.g. malformation, growth and bioaccumulation) were recorded simultaneously during sediment exposures. In addition, several analytical approaches were used to assess the bioavailability of these two metals and the correlation with observed toxic effects. It was observed that embryos and larvae had different toxic responses to these metals with embryos having higher tolerance than larvae. For the metal concentrations (Cu and Pb) in sediment higher than 300 mg/kg each, the survival rates of larvae were significantly lower than that of the control group. In addition, the larval morphology (i.e. body length and body weight) decreased with increased metal concentrations. The heavy metals were also bioaccumulated in larvae with increased concentration in sediments. We will further evaluate whether Chelex-100 resin extraction method can be an effective method in predicting the bioavailability of sediment Cu and Pb to medaka fish.

TH010

The ecotoxicity of Emamectin Benzoate to benthic marine organisms

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Emamectin Benzoate (EMB) has been authorised in Scotland for use as a veterinary medicine to control sea lice in farmed salmon for almost 20 years. EMB partitions rapidly to sediments and, in the marine environment, its mode of action targets crustacean sea lice species. The non-target species expected to be most affected by environmental EMB exposure are therefore benthic crustaceans living near salmon sea pens. While short term sediment ecotoxicity data on marine organisms are available for EMB, it has not been possible to develop a reliable sediment Environmental Quality Standard (EQS) for benthic marine organisms because the only long-term sediment ecotoxicity data available was for a freshwater species (*Chironomus riparius*) and potential differences in sensitivity to EMB between marine and freshwater benthic species could not be resolved. In order to fill this data gap in the EMB ecotoxicity dataset, a programme of new long-term testing with marine sediment species was carried out during 2017/2018, which comprised the following tests: 10-day static toxicity of EMB to the lugworm (*Arenicola marina*) (endpoints covering mortality and feeding). 28-day semi-static toxicity of EMB to the amphipod (*Leptocheirus plumulosus*) (endpoints covering mortality, growth and reproduction). 75-day semi-static toxicity of EMB to the amphipod (*Corophium volutator*) (endpoints covering mortality, growth and reproduction). All of the studies involved the exposure of test organisms directly to sediment spiked with EMB, and the chemical verification of sediment EMB exposure concentrations. The results of these studies indicated that EMB was much less toxic to marine benthic organisms, and particularly benthic crustaceans, than expected. Mean measured sediment ‘no effect’ concentrations ranged from 12.9 µg/L for 10-day *Arenicola marina* feeding behaviour (casting) to greater than 61.3 µg/L for 75-day growth and reproduction in *Corophium volutator*. We will present the full results of this sediment ecotoxicity testing programme, as well as discussing the implications of the results with respect to the derivation of an updated sediment EQS for EMB.

TH011
Identifying non-target toxicants in sediment using effect-directed analysis with a multiple-level endpoint approach
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Identifying causal relationship between sediment contamination and ecological responses is challenging. Bioaccessibility-based XAD extraction and multiple cell-based bioassays were incorporated into effect-directed analysis (EDA) to identify sediment-bound toxicants, with a special consideration of bioaccessibility and toxicity pathways. The XAD extracts of sediment samples from urban waterways in Guangzhou, China were screened using cell viability bioassays with cell lines from four organs. Pronounced effects were noted in the bioassays with SH-SY5Y and MCF7 cell lines, thus neurotoxicity and estrogenic effects were focused in next-step EDA using 35 normal phase liquid chromatography fractions. In addition to cell viability, reactive oxygen species and estrogenic receptor activity were applied as subcellular endpoints for neurotoxicity and estrogenic effect pathway directed bioassays, respectively. Suspect toxicants were identified in active fractions using GC/MS. Toxicity confirmation was performed by evaluating toxicity contributions of identified toxicants to proposed pathways. Cypermethrin and fipronil sulfone were found as the major contributors to neurotoxicity, while nonylphenol explained most of estrogenic activity in the test sediments. The finding of neurotoxicants was consistent with previous *in vivo* EDAs in the same area, suggesting that considering bioavailability and toxicity pathways in screening testing improved environmental relevance for *in vitro* EDA.

TH012
Developing novel sediment toxicity bioassays with Hediste diversicolor polychaete coelomocytes
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The contamination of estuarine sediments after decades of uncontrolled industrial exploitation and urban wastes release is a critical issue that can affect ecosystems health. The ancient pollutants in sediments can pose an environmental risk especially when the physico-chemical conditions of sediments are altered. Hence, a proper assessment of the potential adverse effects of contaminated sediments is crucial. Nowadays, the study of the pollutant levels in sediments is receiving increasing attention, but still the information regarding their effects is scarce. These effects could be assessed by means of toxicity bioassays applied in ecologically relevant species. Among them, the polychaete *Hediste diversicolor* has been characterized by its abundance in sediments and by its capacity to accumulate pollutants. Therefore, it could be considered as a sentinel organism for sediment toxicity assessment. In addition, coelomocytes have become a target system in ecotoxicology due to their capacity to predict impairments caused by pollutants at longer exposure levels. The aim of this work is to develop accurate sediment toxicity test with the coelomocytes of *H. diversicolor*. First, cell retrieval procedures and coelomocyte subpopulation identification were addressed. Primary cultures of coelomocytes were exposed to a range of model (Triton X-100, $CdCl_2$) and novel (Ag NPs) pollutants, and to elutriates coming from real sediments (collected in Bilbao estuary). After different time exposures (1-24h), coelomocyte viability (Calcein-AM) and mortality (PI) were assessed through microplate and flow cytometric analysis. Coelomocytes were extruded by electric stimulation (9V) using PBS adjusted to the salinity of ragworms coelomic fluid (0.17M NaCl) and passed through 40µm strainer to avoid gametes. Coelomocyte population was composed by two major cell groups, amoebocytes and eleocytes. Both subpopulations showed gradual responses after exposure to the different pollutants, being Calcein AM viability test more sensitive and responsive endpoint than IP. According to the cytotoxicity measured in coelomocytes and to the chemical analysis performed in the elutriates of real sediments, a pollution gradient of the sampling points was established. The obtained results prove the usefulness and the potential of *in vitro* toxicity test with *H. diversicolor* coelomocytes for the assessment of polluted sediments.Ack: Basque Gov (IT810-13), Univ. Basque Country and MINECO (CTM2017-87766-R).

TH013
Toxicity evaluations of sediment collected in the Osaka Bay, Japan using medaka embryos
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Chemicals discharged through the human activities have been carried to the sea area by river water, rainfall, and other factors, and then are settled on sediments finally. They have been possibly affecting benthic animals individually or multiply. But their actual conditions in the field are almost unknown. We have been developing new method using fish embryos for the evaluation of sediment toxicities collected from the field. Because the embryos are kept on the polluted sediment with slight amount of pore water and without overlying water, this method can evaluate the toxicities in sediment alone. Osaka Bay is located at the eastern end of the Seto Inland Sea, and is enclosed by Osaka and Hyogo Prefectures, which are a couple of the most densely populated areas in Japan. The bay is surrounded by the island of Honshu and Awaji Island, and is classified as an enclosed coastal sea with only two narrow entrances. This limits water exchange in the bay, and possibly leads to increase risk of marine pollution with heavy traffic together with the presence of large industrial plants. There have been no examinations on the sediment toxicities obtained from the Osaka bay to aquatic organisms. In the present study, we collected the field sediments over 20 locations along the shoreline of the Osaka bay from 2014 to 2016, and evaluated the sediment toxicities using the embryos of Japanese medaka (*Oryzias latipes*). The dried sediment samples were rinsed with an embryo raring mediums. Ten embryos were embedded in half of them in the each sediments, which is laid at a glass petri dish with 35 mm of diameter, and were developed on the sediment in an incubator set at 23°C for 7 days post-fertilization. In the period, the rearing water was not added to the dishes. Three dishes contained the sediment were prepared as sampling site (n=3). An embryo was then individually transferred in a hole of 48-well microplates containing dechlorinated tap water, and kept until 3 days after hatching. The toxicities were evaluated with their developing condition, mortality, hatching days, abnormality in embryo, and malformation in hatching larvae. Only 60% embryos exposed to sediments collected at the mouth of Yodo river were hatched. Their mortality was only 3%, but they had not been able to hatch. For other sites, we could not almost observe the deformity in the embryos and hatching larvae. However, sediments from some sites decreased hatching rates and delayed their hatch.

TH014
Assessment of chronic toxicity of REE-enriched sediments from a prospective mining area on Myriophyllum alterniflorum
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Rare earth elements (REE) are strategic compounds increasingly used in emerging technologies. The future exploitation of areas with high REE contents play a strategic geopolitical role around the world. Opening new mining areas contribute to increased REE mobility and will influence their fate in the environment. Hence,

establishing baseline ecotoxicity of REE in prospective mining areas will be crucial to monitor the potential REE enrichment in the surrounding environments. Despite the growing number of studies on REE toxicity, their effects on aquatic organisms is not fully understood. Moreover, their effects on organisms living in the sediments and more specifically aquatic plants, remain scare. Hence, the aim of the current study is to assess the chronic toxicity of naturally REE-enriched sediments on the submerged growing *Myriophyllum alterniflorum.* Every two weeks, effects on growth, pigment and phenolic compound content and dry matter content are assessed along with uptake and translocation of REE to various plant parts. The experiment is performed in a water-sediment system. Six cm-long apical shoot sections of *M. alterniflorum* from axenic cultures are used as model organisms for the present study. Plants are exposed to two REE-enriched sediments for 6 weeks; namely N2 and B4, and a control sediment established based on the characteristics of the natural sediments. N2 and B4, were collected in North Quebec, Canada. They were selected based on their parent materials. The physico-chemical analyses on sediments showed that B4 contains the highest amount of REE and has the highest fine grain fraction. The first results on exposed plants show that both sediments lead to a growth inhibition in term of length and fresh biomass. Interestingly, the plants exposed to REE-enriched sediments present longer roots than those of the control sediment. This could be linked to a lack of nutrients in the contaminated sediments, or interactions between REE and plant nutrients. These results may also indicate a phyto-available fraction of REE from the sediments that leads to significant changes in plant growth behavior, and may also hint on a potential transfer of REE through the food web chain. Further analyses are ongoing to better define the baseline ecotoxicity of REE on *M. alterniflorum*. The present study provides first insights on REE behavior between sediments and plants, and their potential chronic toxicity on aquatic organisms around prospective mining areas.

TH015

Microbiological biotests for sediment risk assessment - A question of reproducibility and reliability

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Use of microbiological biotests in sediment risk assessment is controversial in Europe. While some countries have implemented them in their decision making frameworks, managers in other countries are doubtful regarding reliability and certainty of ecotoxicological results, and to what extent they can support well-informed decision. Questions that are often raised are those on reproducibility of biotest results and their variability compared to chemical data. In this study, data are gathered on sediment cores that were taken biweekly at two sites in the Elbe river catchment. Analyzing the ecotoxicological depth profile with different test systems were supposed to provide information on a)Reproducibility of ecotoxicological answers to sediments collected at different times from the same site, and b)variability of biotest data and reliability of the different biotests with regard to ecotoxicological thresholds. Sediment cores of up to 80 cm length were taken 3 times every 2 weeks from the same location. The core was divided horizontally into slices of 1.5 to 3 cm thickness. Analysis: Vertical element profiles were determined by portable XRAF to identify comparable layers in the various cores from the same location. Biotesting: Algae growth inhibition assay with *Raphidocelis subcapitata* (AGI), luminescence bacteria test with *Allivibrio fischeri* (LBT) (both tests on elutriates) and the bacterial sediment contact assay with *Arthrobacter globiformis* (BCT) were carried out applying a miniaturized protocol. Locations: a) the "Alte Süderelbe", a sidearm of the Elbe river which is not tidally influenced, Febrary/March 2017. b) a small harbor upstream of Hamburg (Hohendeicher Hafen), September, October 2018. Samples were taken from a wooden swimming pier without boat traffic Currently, only the study of the Alte Süderelbe is completed. Results from Hohendeicher Hafen will be finalized by December 2018. Ecotox data from Alte Süderelbe sediments showed good agreement between the different corer and point towards good reproducibility of AGI and LBT data. (BCT was only performed on Hohendeicher Hafen sediments). There were, however, strong differences in the data on the upper sediment layer that may be due to disturbances of the surface during sampling which had been done wading into the river. For the second study, samples were taken from the pier, reducing the amount of resuspension. Data from both sites will be shown and discussed at the conference.

TH016

SEDIMENT RESUSPENSION EVENTS - THE IMPACT OF CLIMATE CHANGE ON SEDIMENT-BOUND METALS: Toxicity

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Association of trace elements to particulate matter reduces bioavailability and hence toxicity for most metals. However, investigations into the fate and effect of metals associated with particulates during erosion or resuspension events (e.g. during heavy rainfall) is lacking. Future climate change scenarios predict more frequent and intense precipitation events that could lead to remobilization of sediments and an increase in metal dissociation from particulate matter resulting in increased bioavailability and toxicity for aquatic organisms. Sudbury, Ontario has been exposed to decades of smelter emissions that have released metal(loid)-bearing particulate matter, which has accumulated in lake bottom sediments. The present study aimed to investigate the lethality and bioavailability of metals to aquatic invertebrates following exposure to field collected water and sediments samples from Sudbury area lakes. To assess toxicity, a novel sediment resuspension test was developed to measure lethality and bioavailability of metals to *Daphnia magna.* In addition, a standard *Chironomus dilutus* sediment test was conducted to compare toxicity and bioavailability. Results showed that survival rates differed amongst sites and that bioaccumulation of certain metals varied between tests and sites. Results suggest that sediment resuspensions can affect metal bioavailability, resulting in increased bioaccumulation and toxicity in aquatic primary consumers. Based on these preliminary results, further investigation is needed to better understand the environmental impact of climate change on contaminated sediments.

TH017

Impact of agricultural practices on sediment quality of small streams in Switzerland

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In Switzerland, the National Surface Water Quality Monitoring Program "NAWA" documents and evaluates the status and development of surface waters. This program includes a large network of monitoring stations for long-term continuous measurements (TREND) and a focused program at selected sites (SPEZ). In the framework of the NAWA SPEZ program, two studies on the contamination of small to medium sized streams by plant protection products were conducted in 2012 and 2015 [1,2,3]. The first campaigns showed that the concentrations of several insecticides, herbicides and fungicides measured in the surface water exceeded the corresponding environmental quality standards [2,3]. Based on physico-chemical properties of some plant protection products (low water solubility, hydrophobicity) it is highly likely that they are present in sediments too. However, the occurrence and concentrations of pesticide residues in sediments of surface water bodies in Switzerland and their impact to the benthic fauna and flora are yet largely unknown. In parallel to the NAWA SPEZ project 2017, which studies surface water quality in relation to the application of plant protection products in five small streams surrounded by agricultural land, the Ecotox Centre studied sediment contamination and the potential adverse effects of these contaminated sediments using a battery of sediment-contact tests. Considering potential temporal variability in pesticide use, the study was conducted during a whole season, from March to October, with monthly sampling. The concentrations of several plant protection products targeted in this study exceeded existing sediment quality guidelines, indicating a potential risk to benthic invertebrates in sediment and suspended matter samples collected for at least one of the sampling months. Depending on the sampling site the mixture risk was mainly dominated by the organophosphate insecticide chlorpyrifos or by pyrethroid insecticides. The results of the toxicity tests showed adverse effects on benthic organisms (*H.incongruens, C.riparius, C.elegans*) at some sites but the measured endpoints showed large temporal variability for a same site. [1] BAFU 2013: NAWA – Nationale Beobachtung Oberflächengewässerqualität. Konzept Fliessgewässer. Bundesamt für Umwelt, Bern. Umwelt-Wissen Nr. 1327: 72 S. [2] Wittmer et al. (2014). Aqua & Gas, 94(3), 32-43. [3] Doppler et al. (2017). Aqua & Gas, (4), 46-56.

TH018

Reducing uncertainty in sediment contact testing by considering natural variability and harmonizing control sediments

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Introduction: Sediment contact tests (SCT) aim to assess the toxicity of anthropogenic contaminants in whole sediments introduced into water bodies. However, environmental samples do not only differ in their quantity and quality of contamination, but also in terms of their geochemical properties, such as grain size distribution or content of organic matter. These sediment properties might also affect the test organisms and thus impede the interpretation of toxicity data, e.g. by producing false-positive results. This has already been shown for various benthic organisms in freshwater sediments (Ankley et al. (1994) Environ. Toxicol. Chem. 13: 627–635; Höss et al. (2010) Environ Poll 158: 2999–3010). Attempts to account for the variability of test results of SCTs in form of thresholds or corridors for tolerable inhibitory effects (Höss et al. (2010) Environ Poll 158: 2999–3010) might lead to false-negative results and an underestimation of risk. Therefore, we want to present a strategy, how to reduce uncertainty in sediment contact testing for a more reliable risk assessment. Methods: Nine different SCTs

(*Arthrobacter* test, ISO/CD 10872; *Myriophyllum* test, ISO 16191; *Heterocypris* test, ISO 14371; *Caenorhabditis* test, ISO 10872; *Chironomus* test, AFNOR T90-339-1; *Hyalella* test, ISO 16303; *Tubifex* test, OECD 225; *Danio* test, ISO 15088, adapted) with organisms from various trophic and organizational levels will be applied to a selection of min. 30 reference sediments, in order to investigate the species-specific variability due to differences in geo-physico-chemical properties of lowly contaminated freshwater sediments. The project comprises two work packages: In WP1, a common field-collected sediment will be defined as control sediment for all test systems. In WP2, min. 30 lowly contaminated sediments from rivers and lakes will be tested with all sediment contact tests. Sediments will be selected according to following criteria: sites should be in a (very) good ecological and chemical status; chemical contamination is below 2 x TEC for each substance (SQGs according to De Deckere et al. (2011) J Soil Sed 11:504-517); sediments show a gradient of texture and organic content. Variability of test results will be analyzed statistically in order to derive reliable and safe toxicity thresholds. Aimed Results: (1) A common natural control sediment will allow comparison of test results among all SCTs. (2) Revision of endpoint-specific toxicity thresholds will ensure environmental safety.

TH019

Contaminated marine sediments in the Nordic countries; review of remediation approaches. Report from the CONSENS project

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Introduction: For the last two decades, an increasing awareness of legacy contaminated sediments has been seen. Contaminated sediments have been recognized as a source of contamination in aquatic food chains, and the concerns regarding ecological and human health risks continue to grow. Worldwide, a number of sites have been identified and assigned priority for remediation. However, governance and regulatory instruments vary between countries. The Nordic Council of Ministers have recognized the need to compile knowledge of remediation strategies and to evaluate the different approaches within the Nordic countries, to ensure that the best possible measures are taken in the future. A new report presents a status of regulatory framework and sediment management in Norway, Sweden, Finland and Denmark. Further, the report gives an overview of remediation approaches, and presents experience from some well-documented remediation sites within the Nordic countries. **Methods:** A literature review has been carried out by a group of Nordic scientists, which allows for a unique comparison between the Nordic countries. Information and data on regulations, contamination and remediation are not easy obtained as most reports and official documents have been written in Nordic languages, and information can be spread out between many government authorities. Typically, surveys and monitoring of remediation sites are not published in scientific peer review journals. **Results and discussion:** The review shows the inequality in national remediation strategies and prioritizing between the Nordic countries. While Norway has included contaminated sediments in the country's priorities since almost 30 years, Finland has not yet started any remedial action on polluted marine sediments. Remediation approaches includes traditional methods like dredging that has been extensively used for navigation purposes and to maintain sailing depths whereas isolation capping has been conducted in a few sites in Norway, and is under consideration in Sweden. The use of active materials is still in it's infancy. There is not a lot of documentation on post-remediation monitoring, though this phase appears to be very important in order to evaluate the efficiency and improve the techniques. Criteria for deciding upon remediation approaches should include efficiency, adverse effects, costs and benefits of the different approaches.

TH020

Establishing Cleanup Levels for Contaminated Sediment Sites in Norway Based on Bioavailability

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Introduction Traditionally, cleanup strategies have focused on removal or capping of contaminated sediments in order to reduce the sediment concentrations within the biologically active zone, even though the risk for exposure is more related to porewater concentrations than to total sediment concentrations. Recently, activated carbon (AC) and other active materials have been shown to reduce the bioavailability of various contaminants in aquatic sediment, measured as reduced porewater concentrations and reduced flux from sediment to water. The use of AC may reduce the thickness of the capping layer to a few centimeters (thin-layer capping), thus also potentially reducing remedial action costs. However, the use of AC may not meet standard sediment cleanup goals as AC does not destroy contaminants, it sequesters them. This paper presents three examples of Norwegian remedial action plans where proposed sediment cleanup levels have been based on reducing bioavailability rather than sediment concentrations. Materials and methods For all three sites, the proposed cleanup levels are based on bioavailability (porewater concentrations) and ecological risk reduction rather than total sediment concentrations. In the Mosjøen site, the remedial action was completed in 2017; action is pending at the other two sites. The approach for assessing risk, establishing site-specific cleanup levels and assessing remedial technology feasibility is presented. Results and discussion Tier 1 sediment cleanup levels established in Norway are based on screening levels ranging from natural background to severe impairment. Cleanup levels are usually set to level 2. Higher cleanup levels can be accepted if Tier 2 and 3 studies are performed; Tier 3 cleanup levels are based on bioavailability and site-specific conditions. In the Mosjøen site, Norwegian authorities have accepted a Tier 3 sediment cleanup level that is based on site-specific partitioning studies and is protective of porewater and surface water quality. However, for the two other sites Norwegian authorities have not yet accepted proposed similar approaches, which focus on risk reduction and the use of thin AC capping as a thick isolation cap alternative. Conclusions Potential ecological impacts associated with different remedial technologies must be taken into consideration when proposing cleanup strategies focused on risk reduction.

TH021

Laboratory and Field-Based Assessment of the Effects of Sediment Cover Materials on Zinc Flux, Bioavailability, and Toxicity

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A former mining site in the southern United States has been the subject of extensive remediation and restoration for the past few years, with significant focus on disconnecting mine spoils from groundwater and managing the quantity and quality of runoff. An investigation was conducted to compare the efficacy of selected cover materials for decreasing Zn dissolution during periods when the hypolimnion is anoxic and acidic (pH=5.5). Cover materials were selected based on laboratory batch testing and included gravel, AquaBlok, limestone, and limestone + bonechar, gravel + limestone, and gravel + bentonite clay + limestone. Field studies employed Limnocorrals (LC) to isolate the water columns above various cover treatments. Simultaneous in-situ and ex-situ toxicity tests were conducted using *Daphnia magna*, *Hyalella azteca*, and *Chironomus dilutus*. Test organisms were protected from temperature shock by pre-acclimating over 24 h and then deploying the test chambers in a Toxicity Assessment Container System (TACS). Test organisms were exposed to surficial sediments and overlying water in the reference (no cover) LC and in LCs containing surficial sediments plus cover materials. Ex-situ testing was conducted in waters and/or sediment cores collected from adjacent each LC. These exposures were conducted at corresponding in-situ temperatures (15 to 19 °C). Laboratory tests involved a series of acute to short-term chronic toxicity tests using site waters and sediment cores. Results from in-situ testing demonstrated the usefulness of the TACS and provided similar results to the ex-situ testing. Overall, there were no differences in biological responses/endpoints between treatments involving sediment cover materials, and this was true in both the field and laboratory tests. This was likely due to the fact that dissolved Zn in the surface water were below-threshold levels. All cover materials successfully reduced Zn release from the sediment, although some may be less effective under certain hydrologic conditions. While gravel + limestone stopped Zn dissolution, the addition of bentonite clay created an additional impermeable barrier. Gravel + clay + limestone were the optimal cover for controlling pH and restricting Zn dissolution/release. This field and lab based weight-of-evidence study provided for more effective decision-making, with reduced uncertainty, than standard laboratory and chemistry-only approaches.

TH022

Adverse biological effects of activated carbon amendment are dependent on particle size and the test organism

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Thin-layer capping with activated carbon (AC) is an *in situ* method for remediation of contaminated sediments. Though efficient in binding hydrophobic organic contaminants and reducing bioavailability, studies have reported adverse effects on benthic organisms, such as weight loss, reduced feeding rates, and mortality. These effects have been associated with dose and particle size of AC, with finer particles and higher doses causing more severe effects. The aim of this study was to compare adverse effects of powdered AC (PAC) and granular AC (GAC) on two deposit feeding marine benthic invertebrates: the bivalve *Limecola balthica* and the polychaete *Marenzelleria spp*. Five different particle sizes, ranging from 15 μm to 1700 μm, were tested: three PACs and two GACs. Both organisms are capable of ingesting PAC, but not GAC. Organisms and sediment were collected from a non-polluted site in the Baltic Sea. Thin layer caps (ca 2 mm) of AC were applied at a relatively low dose (600 g?m^{-2}), and after 3 months, animals were fed a pulse of ^{14}C-labeled diatom plankton, then gut purged, weighed for dry weight, and measured for ^{14}C uptake and lipid content. A significant loss of weight and strongly reduced ^{14}C uptake occurred in the polychaetae *Marenzelleria spp*. in response to all three PACs. There were, however, no increase in mortality or decrease in lipid content. GAC had a positive effect on ^{14}C uptake by the polychaete when GAC treatments were pooled and compared statistically to control. AC was not observed to affect the bivalve *Limecola balthica*: neither PAC nor GAC treatments significantly affected survival, dry weight, ^{14}C uptake, or lipid content of the bivalve. Our results confirm previous studies on freshwater species, which have found that adverse effects are dependent on particle size of AC. Furthermore, we show that effects are species-dependent and connected to ingestion: the polychaete was strongly adversely affected by ingestible powdered AC, whereas non-ingestible granular AC had some positive effect. This study highlights the risks to benthic communities when using powdered AC for sediment remediation and the importance of selecting appropriate test organisms when evaluating biological effects on benthic organisms. Results from this study strengthen the hypothesis that adverse effects of AC on benthic organisms are caused by reduced feeding or reduced assimilation of food by the organism, rather than nutrient sorption to AC outside the organism.

TH023
SETAC Sediment Interest Group
S.M. Bay, Southern California Coastal Water Research Project / Toxicology
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The Indoor Environment: Emerging Contaminant Identification, Analysis and Quantification for Exposure Assessment (P)

TH024
Analyses of chlorinated paraffins and pesticides in the indoor environment for human exposure assessment
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Daily, people spend a lot of time indoors, either at work in offices or at home. In the indoor environment they are exposed to a variety of compounds, released from furniture, carpets, electronic devices, insulation or construction materials by a combination of evaporation and abrasion. Within the SHINE (Target and non-target Screening of cHemicals in the Indoor enviroNment for human Exposure assessment) project, target analyses and non-target screening were performed on indoor dust and air samples for the presence and concentrations of emerging compounds of different compound classes, such as phosphate flame retardants (PFRs), new and legacy brominated flame retardants (BFRs), chlorinated paraffins (CPs), hexabromocyclododecanes (HBCDs), per- and polyfluoroalkyl substances (PFASs), novel plasticizers and pesticides. These results will be used in existing human exposure models to predict the internal human exposure for a set of emerging substances in EU populations. Here, the results for concentrations of 15 pesticides, and short-, medium- and long chain CPs in dust samples from daycare centers, offices and houses from Sweden, Ireland, Belgium and The Netherlands are presented. Results showed that pesticides as well as short, medium and long chain CPs were detected in indoor dust. The highest concentrations of pesticides were found for permethrin, which had also the highest detection rate. The highest concentrations of CPs were detected for MCCPs. Dust from houses of The Netherlands were less contaminated with pesticides and CPs than houses from Belgium, Ireland and Sweden.

TH025
An assessment of the exposure of the Irish population to selected brominated flame retardants via indoor air and dust
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Polybrominated diphenyl ethers (PBDEs), hexabromocycododecane (HBCDD)–POP-BFRs –and decabromodiphenyl ethane (DBDPE) were measured in air and dust samples from Irish indoor microenvironments (MEs). HBCDD and PBDEs were used as flame retardants in a variety of soft furnishings, building insulation foams, electronic and electrical goods. Human exposure to POP-BFRs have been shown to cause many adverse health effects and coupled with evidence of their bioaccumulation potential and persistence, has resulted in their ban or use restrictions under the Stockholm Convention. Legislative restrictions have created a market demand for replacement flame retardants, such as DBDPE. This study reports for the first time the estimated exposure of Irish adults and toddlers to POP-BFRs via dust ingestion and air inhalation from several MEs. Samples were collected from Irish homes, cars, offices and primary school classrooms (n=30 per ME) between 2016-2017. Air samples were collected using a sorbent (XAD-3) impregnated polyurethane foam disk, extracted via pressurised liquid extraction using an ASE-350. Dust samples were collected by vacuuming floor surfaces, extracted via a combination of vortexing and ultrasoncation and purified on SPE cartridges, concentrated and analysed via GC-EI/MS or LC-MS/MS. Dust samples collected from Irish offices and schools contained relatively low concentrations of BDE-209 (median: 3500 ng/g; median:8100 ng/g resp.), compared to levels in homes and cars (median: 13000 ng/g, median: 26000 ng/g resp.). Highest concentrations of DBDPE were observed in schools (median: 10000 ng/g). The highest BDE-209 air concentrations were detected in samples collected from schools (median: 410 pg/m^3), followed by homes, offices and cars (median: 410 pg/m^3; median: 240 pg/m^3; median: 200 pg/m^3 resp.). The highest concentration of DBDPE in air samples (7000 pg/m^3) was detected in homes. This study reports concentrations in indoor air and dust of DBDPE and to the authors' knowledge the first air concentration data for PBDEs, HBCDDs, and DBDPE in primary school class rooms. Concentrations of DBDPE in dust samples for all our studied MEs are substantially higher than those previously reported internationally.Our data suggests the widespread use in Ireland of BDE-209 and its replacement DBDPE. Estimates of exposure via air inhalation and dust ingestion for both Irish adults and toddlers to DBDPE and BDE-209 exceed reported exposure estimates for the UK population.

TH026
Evaluating the RAIDAR-ICE model with monitoring and biomonitoring data
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Humans are exposed to a wide variety of chemicals that originate in "near-field" environments (i.e., indoors) as a part of building materials, consumer goods and articles, and products used daily, e.g., household products, personal care products. Measured data are emerging through various monitoring and biomonitoring projects and programs. For example, the Cefic-LRI SHINE project is conducting targeted and non-targeted analyses of dust and air samples in indoor environments coupled with a literature review for biomonitoring data for the target chemicals (e.g., flame retardants, pesticides, plasticizers). Mechanistic, mass-balance, multi-media models serve as powerful tools to understand important exposure pathways and to predict exposures in the absence of measured data for human health assessment. Such models that link external exposures with internal exposures can also be used to integrate monitoring data (air, dust, surface concentrations) with biomonitoring data (blood, urine). There is a need to test (evaluate) exposure models to foster confidence in their application in regulatory programs in both data rich and data poor contexts. The Risk Assessment IDentification And Ranking – Indoor and Consumer Exposure (RAIDAR-ICE) model combines and indoor fate model with a toxicokinetic model to simulate chemical exposures from direct (e.g., personal care products) and indirect (e.g., vaporization of chemicals from building materials) sources of chemicals. It simulates human exposure from the near-field environment through inhalation of indoor air, dermal absorption, non-dietary object-hand-mouth contact. In this study we evaluate the RAIDAR-ICE model with existing and emerging monitoring and biomonitoring datasets for a range of chemicals, including new data obtained in the SHINE project. The case study chemicals comprise a broad range of use categories, partitioning properties, and biotransformation half-lives.

TH027
Li-BIM, an agent-based model to quantify human exposure variability in residential indoors
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New building design and renovation standards tend to increase airtightness and use of newer materials to meet current environmental challenges. This leads to confined spaces in which building chemicals that have been released in the indoor air of the dwelling accumulate. Their residence time is highly affected by physical removal from occupants activities such as vacuum cleaning, windows opening. Occupants are exposed to indoor chemicals through near-field exposure pathways that affect significantly the indoor fate of chemicals. Therefore, human exposure and chemical fate concerns should be addressed together with the question the occupants as a contributor for and a receiver of indoor pollution. This approach implies a substantial focus on the heterogeneity in human profiles and activities which are key drivers for quantifying human exposure in indoor environments. Statistical analysis from biomonitoring data or field surveys can provide valuable information to account for exposure variability. However, the lack of data for the vast majority of chemicals or a wide range of households' archetypes turns out to be a stumbling block for achieving accurate exposure prediction. To faces these limitations, Artificial Intelligence techniques are promising in describing

dynamics mainly driven by human behaviors and among them, the agent-based approach is particularly well suited to model human cognition. Therefore, we propose an agent-based modeling of the building-occupants' interaction to assess the impact of the occupants' behavior on the chemicals fate as well as the occupants' exposure to the resulting indoor chemicals. The model is structured around an agent-based model, Li-BIM (Live in BIM), simulating the occupants' behaviour in residential dwellings. A twofold scale has been set up at the household and the individual level to characterize the occupants with a set of attributes likely to influence their activity pattern and physiological parameters. Each of the resulting 270 profiles and their indoor exposure is simulated during a whole year in a low-energy dwelling. At the time of the conference, K-mean clustering algorithm will be applied to identify several households' archetypes representative of the exposure variability. This study will offer a better understanding of exposure patterns and paves the way to support the design of healthier building indoors with targeted guidance to specific households' archetypes.

TH028

Higher levels of halogenated flame retardants on handheld than non-handheld electronic devices

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Flame retardants (FRs), particularly halogenated flame retardants (HFRs) have been widely added to electronic products and are consistently detected in indoor environments around the world. FRs move from products to dust, air and also to humans, and many have evidence of toxicity. It is thus important to better understand the factors controlling FR emissions from consumer products, FR distribution in the indoor environment, and the resulting human exposure pathways. Here, HFRs were measured in floor dust, air and surface wipes of electronic devices, and wipes of participants hands in 51 Canadian homes. Target compounds were 14 polybrominated diphenyl ethers (PBDEs) and 10 novel halogenated flame retardants (NFRs). Ancillary data on house characteristics, participant lifestyle and electronic products were also collected. Our goals were to: 1) evaluate the relationship between HFR levels in the various indoor matrices; 2) identify differences in distributions and patterns of legacy (e.g., PBDEs) vs. their replacement NFRs; 3) evaluate associations between measured indoor levels with information on housing characteristics and consumer product use; and 4) to assess exposure routes of HFRs to humans. Hand-held electronics (e.g., tablets and cell phones) had higher concentrations of a wide range of HFRs as compared to non-hand-held devices and none had a distinct profile, suggesting that their surfaces are behaving both as a source of FRs and a sink for HFRs from other indoor sources. DBDPE, followed by BDE-209 were exceptions with the highest concentrations in TV wipes, which is consistent with more stringent flammability standards for these devices. The number of TVs in the household correlated with the mass fraction of DBDPE in dust; lower levels were found in homes with no TV, indicating the impact of TV flammability standards. HFR concentrations on hands were correlated with dust mass fractions, but less so with air concentrations. Several HFRs wiped from hands were correlated with those wiped from laptop surfaces. Hand and dust samples were dominated by BDE47, 99 and 209, and DBDPE whereas air concentrations were dominated by BDE47 and 99, TBP-AE, PBBz, PBT and EH-TBB. Air mixing was related to lower levels of some HFRs whereas higher levels were measured in separate (poorly mixed) zones. These results suggest that handheld devices could be a pathway for exposure and also indicate the impact of higher TV flammability standards on household HFR levels.

TH029

Occurrence of 18 plasticizers and their alternatives in indoor dust from France: levels and preliminary assessment of children exposure.

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Indoor dust is an important sink of semi-volatile organic compounds (SVOCs). Plasticizers, a group of ubiquitous SVOCs, are present in many consumer products, including building materials, household appliances, food packaging, toys, furniture and personal care products. Some of the classical plasticizers have been banned in Europe due to their adverse effects on human health leading to the introduction of alternatives on the market. Human exposure to these compounds is of concern, especially during the critical period of development (early ages). As spending a considerable time indoor, children are often subject to a high risk of chemical exposure, through indoor dust ingestion, mouthing of toys or other consumer products as well as indoor air inhalation. In this context, a selective, sensitive and multi-residue method was developed to analyze 18 plasticizers, including bisphenols, banned phthalates and their new alternatives. Target chemicals in indoor dust samples were extracted using a solid–liquid extraction method and then purified by a solid-phase clean up step. The extracts were then analyzed using liquid and gas chromatography coupled to mass spectrometry (LC-MS/MS and GC-MS). The developed methodology was fully validated and good performances were obtained in terms of sensitivity, precision, recovery (60-100%), and matrix effect (

TH030

Thermal desorption coupled to GC/MSMS for the quntification of organic pollutants in indoor air and dust

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An analytical method coupling thermal desorption and GC/MSMS was developed for the simultaneous quantification of multi-class organic pollutants including pesticides, phtalates, phenols, HAPs and PCBs in indoor air and dust. Indoor ais samples were collected by using Tenax passive samplers on a 15 days basis and indoor dust was sampled by aspiration on a new sampling head aquipped with a SiC foam for the collection of dust and potential release of molecules from dust to air during aspiration. These foam were extracted by Accelarated Sovent extraction by using acetonitrile at 150°C/1500psi and solvent extract was evaporated to & mL into a fume hood. 100 μL of the dust extract was then deposited on a Tenax tube and internal standard and 10 μL MtBSTFA was added as a derivatisation agent for the GC analysis of some pasticides and phenols. Tube was than extracted by thermal desorption and molecules were quantified by GC-MSMS. Passive sampling tubes were introduce onto an empty thermal desorption tube and internal standards and MtBSTFA were added. They were also desorbed by thermal desorption and quantified by GC/MSMS. Application of thermal desorption to indoor dust extracts permits to decrease strongly sensistivity to a factor of about 50 in comparson to liquid injection and the matrix effect is also reduced. With this method, it is possible, by coupling derivatisation with thermal desorption and GC/MSMS, to quantify many different compounds in one run with good sensistivity and specificity. Detection limits about 0.1 ng were achieved for many compounds. Details of this analytical method and its application to real samples were presented.

TH031

SETAC Human Health Risk Assessment Interest Group

S. Turnblom, Oregon Department of Environmental Quality / Western Region Environmental Cleanup

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Wastewater Effluents: How Research Can Improve Risk Assessment and Regulation (P)

TH032

Potential for toxicity and depuration of a dairy wastewater in a basin with different soil uses

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Knowing the environmental risk that the presence of pollutants causes to aquatic organisms can provide information to prevent it. In this work, the acute toxicity and the effect of the depuration of a dairy wastewater in three points of the Pitingui river (Roseira-SP, Brazil), were studied in order to understand the relation between water quality, the potential of depuration of dairy wastewater and its toxicity. The dairy wastewater was collected in a cooperative in Rio de Janeiro (Brazil). The river water samples were collected during the months of March (rainy season) and June (dry season) in three points: P_1 (near to the source), P_2 (close to a slightly urbanized zone with sanitary effluent discharge) and P_3 (close to a rural area with monocultures). The potential toxic of the dairy wastewater was evaluated by acute assays with the fish *Danio rerio,* that were exposed for 96 h (n=2) to different concentrations: 1, 5, 10, 40 and 70% (effluent diluted with river water samples, P_1, P_2 and P_3). The depuration tests were evaluated by the respirometric method using the BOD EVO sensor system (VELP Scientifica, Italy). Dairy wastewater samples were diluted to 5 and 10% with river water samples (P_1, P_2 and P_3). During assays, the system was maintained with 15

measurement points (n=2) by 168 h. The effluent presented high concentration of Soluble Chemical Oxygen Demand, Total Chemical Oxygen Demand and Total Biochemical Oxygen Demand with 1,544 ± 38 mg·L^{-1}, 5,209 ± 113 mg·L^{-1} and 2,540 ± 297 mg·L^{-1}, respectively, and high nutrient concentration with 100 ± 6 mg·L^{-1} of Total Kjeldahl Nitrogen and 33 ± 3 mg·L^{-1} of Total Phosphorus. Acute toxicity was observed only in the samples taken during the dry period: LC50 of 36.36 ± 4.33% and 24.92 ± 2.42% for P_1 and P_2, respectively. The depuration was higher for samples from the dry period than from the rainy period, where the depuration curves for the 10% of dilution presented a significant difference among river water samples. In this case, P_3 and P_2 depuration potentials were higher than to P_1. Thus, dairy wastewater showed high impact manly in the dry period, that P_2 has higher toxicity potential and depuration potential at short time assay, that is, even with higher clearance potential, the remaining concentration in P2 was toxic to fish. These results were obtained probably due to the lower flow of the river, additionally to organic load discharge for P_2 of 30 mg SCOD L^{-1}, which was not observed for P_1 and P_2.

TH033

Impact of a wastewater treatment plant upgrade on amphipods and other macroinvertebrates: individual and community responses.

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Effluents from wastewater treatment plants (WWTPs) are known as major point sources for the release of contaminants into aquatic environments. Particularly municipal WWTPs equipped with secondary and tertiary treatment steps do not or only partially remove micropollutants which can cause short- or long-term adverse effects in aquatic organisms even at low concentration levels. One possibility to reduce the input of micropollutants into the water cycle is the upgrading of WWTPs with an additional purification stage using, e.g., ozonation or powdered activated carbon. The current work is integrated into the BMBF project "SchussenAktiv*plus*", an ecotoxicological research project conducted at two tributaries of Lake Constance, the rivers Schussen and Argen, in Southern Germany. At the Schussen, a powdered activated carbon stage has been established at the WWTP Langwiese, Ravensburg, in winter 2013. Our part of the project focuses on assessing the overall health status of gammarids and the integrity of the macrozoobenthic community in the rivers Schussen and, in parallel, at the river Argen. Investigations upstream and downstream of this WWTP as well as prior and subsequent to the installation of the WWTP upgrade provides information of the impact of the effluent on the test organisms as well as of the efficiency of the new carbon purification step at this WWTP with respect to the health of aquatic wildlife. Our investigations with gammarids showed that the sex ratio and the fecundity had been affected by the effluent of the WWTP Langwiese prior to the installation of an additional treatment step. The WWTP effluent negatively influenced also the macrozoobenthos community with respect to the abundance of all macrozoobenthic taxa and, particularly, the so-called “sensitive” taxa. Subsequent to the upgrade, gammarids collected at the downstream site did no longer differ from gammarids collected upstream of the WWTP with respect to the investigated health parameters. Furthermore, the total numbers of macrozoobenthic taxa and sensitive taxa were found to be increased at the downstream site after the upgrade of the WWTP. On the basis of our results we conclude that additional wastewater treatment on the basis of activated charcoal is highly efficient to reduce toxicity of the WWTP effluent, and thus, is an efficient tool to improve invertebrate health and community integrity, and to sustainably protect aquatic ecosystems.

TH034

Multi-biomarker responses in a common freshwater mussel (Anodonta anatina) after laboratory exposure to industrial wastewater

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The freshwater mussel *Anodonta anatina* is abundant in most Swedish freshwater ecosystem types and is distributed over most of Europe. As a stationary filter feeder, it is a promising candidate for exposure studies both in the laboratory and in the field. In the present study, we chose *A. anatina* as a model for studying molecular stress biomarkers of aquatic pollution. There is a lacking understanding of natural variation in gene expression and enzymatic activities commonly used as biomarkers in other species. To address variation and stress responses in an environmentally relevant exposure scenario, a laboratory experiment was performed. Mussels were collected local-ly in southern Sweden on a location free from point source pollution, and acclimatized to laboratory conditions for four weeks. A 96 h experiment was performed, in which mussels were exposed to wastewater from a Swedish chemical industry (n=16) or to a control treatment (n=16). Digestive glands and gills were extracted, and gene expression and enzymatic activity will be assessed for a panel of stress biomarkers. Preliminary data will address (i) natural variation in gene expression/ enzyme activity, and (ii) biomarker responses to industrial pollution. Results will subsequently be used to design of a follow-up field experiment to test and predict marker responses *in situ*. Ultimately, the aim is to identify biomarkers that can provide early warnings of exposure to pollution as well as be used to predict adverse effects on higher biological levels.

TH035

Developing biomarkers of sewage effluent exposure in the freshwater amphipod Gammarus fossarum

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Chemicals coming from sewage effluents, can be detected in the aquatic environment in very low concentrations. Attempts to monitor dysfunctions in invertebrates using orthologous genes in fish have produced inconsistent results. Gammarids have been shown as very sensitive to pollution. Particularly in Swiss rivers they have been shown to bioaccumulate a wide range of chemicals, both polar and non-polar. However, to date no reliable molecular markers are available in these species. The aim of this study is to provide a set of new transcriptomic markers in *Gammarus fossarum*. After in-field validations, the new biomarkers could be used to evaluate the state of aquatic ecological niches and the efficiency of wastewater treatment plants (WWTPs). Amphipods were sampled from a Swiss freshwater stream in September 2017, using a standard kick-net method. Sampling was performed 50 m upstream and 50 m downstream of the WWTP. For the transcriptomics analysis, 5 distinct pools were used, each consisting of 5 amphipods, as biological replicates. Sampling was conducted for both males and females, to get a total of 20 samples of total RNA. The RNA samples were sequenced by HiSeq 2500 platform. A subsample of amphipods was fixed to record population markers and potential intersexuality phenotypes. A differential gene expression analysis was conducted to identify differentially expressed genes between up- and downstream populations. A chemical analysis of some of the most common pesticides and pharmaceuticals found in river waters, for both surface water and amphipods was also performed using online SPE LC-HRMS. Chemical analysis revealed higher concentrations in water and amphipod samples at downstream site compared to upstream, for most of the chemicals. The sequencing analysis showed a total of 87 and 56 differentially expressed genes between up and downstream populations for males and females, respectively. Despite male and female gene lists showing little overlap, a gene ontology analysis showed an enrichment in stress response genes in both lists. The presence of consistent molecular changes between up- and downstream populations seems evident, suggesting sub-lethal effects on the amphipods, triggered by the complex chemical mixture found in the water stream. None of the molecular changes observed downstream were evident in the population data. Therefore, the relationship between the transcriptomics data and phenotypic data needs to be further elucidated.

TH036

Effects of a Swiss sewage effluent on the population structure of the freshwater amphipod Gammarus fossarum

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The prevalence of chemicals in lotic habitats is a concern, with the main source of these contaminants being effluent from waste water treatment plants (WWTPs). The impact of many chemicals on amphipods has already been identified. However, considering their long and extensive use in ecotoxicology, it is surprising to find that the literature on this aspect appears to be contentious. The reason for this could be attributed to the rate of variability in the biology and ecology of the gammarids species. For example, the differences in environmental conditions among different countries and phenotypic variability in responses to natural and anthropogenic perturbations. The aim of this study is to investigate the changes in population parameters between amphipods sampled up- and downstream of an industrial WWTP. These data coupled with a chemical knowledge of the stream water will give a deeper understanding on the effects on gammarids in response to exposure to specific chemicals. Amphipods were sampled from a Swiss stream in Sep 2017 and 2018, using a standard kick-net method. Sampling was performed 50 m downstream and 50 m upstream of the WWTP. Sampling was conducted for both males and females, to get a total of 609 amphipods in 2017 and 196 in 2018. A chemical analysis of 58 compounds including common pesticides and pharmaceuticals found in river waters, for both surface water and amphipods was also performed in Sep 2017 using SPE-LC-HRMS. Size, sex ratio and number of eggs of the amphipods of both samplings were recorded. No significant differences in any population parameters were found between up- and downstream populations despite previous transcriptomics and metabolomics studies suggesting impacts on the cellular biology of downstream populations. Chemical analyses revealed higher concentrations in water and amphipod samples at downstream site, for most of the chemicals. Higher bioaccumulation factors in gammarids sampled at downstream site for 6 substances were also found. Considering the differences in chemical concentrations between up- and downstream sites, these data show that the effects of a chronic exposure could be not visible simply looking at macroscopic features. In fact, none of the differences observed in a previous transcriptomics analysis were evident in the population data. In addition, environmental conditions in different sampling periods could hide changes in population parameters of amphipods coming from different samplings.

TH037

Cytotoxicity on brown mussels exposed to cocaine environmental concentrations

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Illicit drugs consumption increased worldwide which produce not only social and health problems but might also generate environmental impacts. Such compounds have been identified as contaminants of emerging concern continuously released into the environment via Wastewater Treatment Plants (WTPs). Previous studies showed that cocaine induced negative effects on non-target freshwater organisms. However, there is a lack of knowledge regarding the cocaine concentrations in marine environment as well as toxicity . This study aimed to investigate the lysosomal membrane stability (LMS) on haemolymph of brown mussels *Perna perna*through Neutral Red Retention Time (NRRT) assay to identify cocaine cytotoxicity. Mussels were exposed up to 168h to different concentrations of cocaine (0,2 μg/L; 2 μg/L; 10 μg/L) based on those found in a tropical coastal zone (Santos Bay, Brazil). Our results reported a decreased of lysossomal membrane stability in mussels exposed to all concentrations tested. These data indicate that cocaine constitute a potential hazard for marine biota and suggest the regularly monitoring of illicit drugs in coastal waters. Further ecotoxicological studies are recommended to better understand the mechanism of action of this compound on marine biota.

TH038

Efficiency of three different wastewater treatment techniques to reduce pesticide loads in agricultural waters: an ecotoxicological evaluation with Gammarus fossarum

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Plant protection products (PPPs) that are applied to safeguard yields can unintentionally enter the aquatic environment, for instance as wastewater resulting from the cleaning of agricultural machinery. This may contradict main targets of the EU Water Framework Directive, that aim to increase the overall surface water quality. Different wastewater treatment techniques exist that can be applied at such point sources to reduce PPP concentrations and thus their impact on aquatic ecosystems and the services they provide. As different treatment procedures are available, the selection of an environmental friendly, and efficient method is of high interest. The latter aspect is part of the present work, which is embedded within the EU Interreg project PHOTOPUR. In this context, we evaluated three methods: i) photocatalysis, ii) ozonation, and iii) the application of activated carbon, for their efficiency to reduce the ecotoxicological potential of five model PPPs (myclobutanil, glyphosate, pyrimethanil, folpet and boscalid) by involving a key species in the ecosystem functioning of heterotroph small water systems. For this purpose, the feeding activity of the amphipod shredder *Gammarus fossarum* on preconditioned black alder leaves was investigated during 7d exposures to increasing concentrations of the model PPPs that were previously treated by i), ii), or iii). Using the feeding activity of the gammarids as sublethal endpoint, dose-response relationships for each PPP-treatment method combination was evaluated. Preliminary results indicate, that while all methods tested have a positive effect on the water quality by decreasing the toxicity of the PPPs for the amphipods, efficiency of the methods differed. For instance, for pyrimethanil the highest mitigation potential was demonstrated using activated carbon. The currently ongoing biotests with *Gammarus*, with the remaining (un-)treated PPPs and their mixtures, will provide a comprehensive picture of the methods' efficiencies, which will be additionally supplemented with analytical PPP quantifications.

TH039

Ecotoxicological assessment of freely bioavailable, polar pollutants in Swedish streams

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The impacts of WWTP effluents are widely studied, whereas possible effects caused by effluents emitted from onsite sewage treatment facilities (OSSFs) are mostly neglected up to now. Since about 20 % of Swedish households are connected to OSSFs, we conducted a study with the aim to assess the impact of OSSF effluents on water quality, in comparison to small and large-scale WWTPs. POCIS with Oasis® HLB were deployed at seven sampling sites within the catchment area of the Fyris River, its tributaries, and Lake Ekoln near Uppsala, Sweden. \n Three rural sampling sites were exclusively impacted by OSSFs in tributaries of the Fyris River, whereas the other sites were mainly impacted by WWTPs, and a mixture of other sources. Sampling was conducted in four different sampling campaigns between November 2014 and September 2015 covering four different seasons. Extracts from the samplers were investigated using target analysis for 74 MPs including pharmaceuticals, personal care products, pesticides, artificial sweeteners and perfluoroalkyl substances (PFASs). Cytotoxicity was assessed with the MTT assay, and dioxin-like activity with the micro-EROD activity assay. The lyticase assisted yeast estrogen screen assay (LYES) was used to detect estrogenicity. Eight selected samples were further tested with Danio rerio embryos and larvae for embryotoxicity (FET), for behavior change in a light-transition test, and for oxidative stress in a fluorescence assay. \n Cytotoxicity was only found at one OSSF site in June 2015. EROD-activity was detected in eight samples for different sampling campaigns, including Lake Ekoln estuary, the small-scale WWTP, one OSSF site and the large-scale WWTP. Estrogen activity occurred at the small-scale WWTP and at one OSSF site for different campaigns. No embryotoxicity could be detected in selected samples, but significant changes in behaviour of zebrafish larvae were observed for the small and the large WWTP, two OSSF sites, and the estuary for different campaigns. Finally, oxidative stress was detected for the small WWTP and one OSSF. \n Multivariate statistics revealed a strong difference between the large-scale WWTP and the other sampling sites, with distinct grouping of pharmaceuticals and PFASs. Furthermore, about 50 % of the variance in the substance patterns could be explained by the size of the treatment plant and the water flow rate. This study shows that the emissions of OSSFs can have an adverse effect on the nearby aquatic ecosystem.

TH040

The acute impact of entrainment passage through coastal marine cooling water systems on planktonic organisms

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Many global industries generate heat as part of their intrinsic processes, these include power generation, desalination, oil refineries, steelworks, and the manufacture of chemicals. To remove excess heat, they require industrial scale cooling systems. Water is the most commonly used coolant, often used in a once through system, whereby it is pumped from source into the industrial unit, used as a coolant and is consequently heated above ambient temperatures, before being transferred back to source, thus removing the surplus heat. Of the water-cooled systems, when available, seawater is advantageous over freshwater, due to its increased abundance relative to freshwater, and a general reduction in legislation governing thermal and chemical discharges, which are often present concerning freshwater sources (Pugh *et al*., 2005). Although these systems are protected by various screens, which stop larger organisms and detritus entering the system, small planktonic organisms will likely be entrained into the system. Entrained material typically includes permanent members of the plankton community (holoplanktonic organisms) such as copepods, and temporary members (meroplanktonic organisms) such as fish eggs and larvae. Except for a paper by Bamber and Seaby (2004) there are few peer reviewed studies examining the impacts of entrainment on locally abundant, ecologically and commercially important species, which may be vulnerable to entrainment. Thus, it is often assumed that there will be 100 % mortality of all entrained organisms. The Entrainment Mimic Unit (EMU) simulates, as realistically as is practically possible, the full range of physical and chemical conditions experienced by entrained organisms during passage through a generic once through cooling water system. It exposes test organisms to increases in pressure and temperature, mechanical damage and biocide application. Following passage through the EMU any mortality is determined 24 hours post simulation. Several temperate fish and invertebrate species were selected for EMU studies based upon their relative commercial and ecological importance. These included both holoplanktonic and meroplanktonic organisms, which are likely to be susceptible to entrainment. These studies showed differences in entrainment induced mortality according species, life stage and season.

TH041

Ecotoxicological impact of semi-open horticultural systems on irrigation water quality

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World horticulture is facing major problems due to water scarcity, either by its low abundance or by the degradation of its quality. Inefficient irrigation methods

and excessive use of Plant Protection Products (PPPs) are amongst the main reasons causing this phenomenom. This must be overcome, not only because growers have to maintain their farms profitable in a long term, but also because of environmental issues. Irrigation efficiency is largely improved in closed soilless systems, where drainage is recirculated to further irrigate the same crop. Nevertheless, open (free drainage) and semi-open systems (reuse of drainage to irrigate secondary crops) are still very common and often they even predominate in many regions. This leads to drainage release into the environment, which causes pollution on soil and water bodies since they contain considerable amounts of nutrients and PPPs. Assessing the environmental impact of those systems on irrigation water quality is therefore imperative. Here we assessed the impact of semi-open systems on the quality of drainages collected from a fruit crop (strawberry) and an ornamental crop (rose), through ecotoxicological assays using the aquatic species *Daphnia magna* (cladocerans), *Vibrio fischeri* (marine bacteria) and *Raphidocelis subcapitata* (green algae). Samples were collected immediately after draining at the substrates and from the reservoirs of both growers. High toxicity of the drainages to *D. magna* (immobilisation test) was found for both strawberry (reservoir: $EC_{50\text{-}48h}$=11%; from the substrate: $EC_{50\text{-}48h}$=48%) and rose (from two reservoirs: $EC_{50\text{-}48h}$=18% and 21%; from the substrate: $EC_{50\text{-}48h}$=8%). The algae *R. subcapitata* was generally not affected on its growth, but inhibition was found for drainages from the substrate of rose (EC_{20}=63%). The bioluminescence of *V. fischeri* was stimulated by the drainages from both rose and strawberry cultivation systems. It is concluded that the drainages from soilless systems affect aquatic organisms, but in a variable extent. For a better environmental performance of soilless cultivation, it is stressed here the importance of preventing drainages to be released into the environment.
Keywords: drainage, ecotoxicity, soilless horticulture.

TH042
The ready biodegradability of woven and non-woven wet wipes in aerated and sealed bottle test systems
R. Commander, E. Pratt, Scymaris Ltd / Environmental Fate
In recent years wet wipes have been used as a convenient method of cleaning and disinfecting for a variety of applications (baby wipes, make-up removal, household cleaning), this study looks at whether there is any difference between the biodegradation of woven and non-woven wipes and whether any difference in biodegradation is observed in aerated and sealed bottle ready biodegradation test systems. The OECD series of environmental fate tests have a number of different methods to determine ready biodegradation. Some of the differences are: Aerating test media vs sealed bottles; Biodegradation measurement: CO_2 evolution; oxygen consumption; dissolved organic carbon; Test substance concentration; Test media concentration; Test volume. The OECD 301B and OECD 310 were chosen as both use carbon measurements to determine biodegradation, but the OECD 301B is an aerated test system while the OECD 310 is a sealed test system. Both studies can be continued up to 60 days if 60% biodegradation was not reached within 28 days. Ready biodegradation tests (OECD 301B and OECD 310) were performed using the same batch of activated sludge from a sewage treatment plant treating predominantly domestic sewage to determine whether any difference in biodegradability was observed with an aerated system and closed bottle system and whether there is a difference between woven and non-woven wipes. The tests were performed on dry wipes without solvent to prevent any anti-microbial action from any residual solvent. Study Study type Test substance concentration (mg/L) TFS concentration (mg/L) Test volume (mL) Temperature (°C) OECD 301B Aerated 20 mg DOC/L 30 1 - 3 L 20 - 21 OECD 310 Sealed bottle 20 mg C/L 30 100 mL 20 - 21 We will present the full results of this ready biodegradation testing programme, as well as discussing the implications of the results.
Keywords: Wet wipes; ready biodegradation; waste water; personal care product

TH043
Determination of Heavy Metal Concentration in Treated and Untreated Wastewater from Two Treatment Plants in Maluti-a-Phofung Municipality
M. Moloi, University of the Free State / Zoology & Entomology
Wastewater, the grey and black water from the industry and households, is a complex mixture of heavy metals, organic and inorganic compounds which may be very detrimental to the aquatic systems if not treated adequately. The wastewater treatment plants therefore work to treat and remove these contaminants to create reusable water that can re-join the water cycle with minimal impact on the environment. Over the years, the status of the wastewater treatment plants in South Africa have deteriorated thereby compromising the quality of the receiving streams and their ecosystems. This study therefore focused on assessing the wastewater treatment works in the Maluti-a-Phofung area for heavy metal pollution of the treated effluent. The water was sampled from the Phuthaditjhaba WWTP and the Harrismith WWTP and analysed for heavy metals (Fe, Mg, Mn, Zn, Cu, Cr, Ni, Pb, Co, As, Cd) using Induced Coupled Plasma Mass Spectrometry (ICP-MS) with LGC Multi-element Standard as the quantifying standard. Results showed that there was no significant difference ($P > 0.005$) in the concentration of heavy metals in influents when compared with effluents for both water treatments plants, indicating the inability of both treatment plants to properly remove metal contaminants. Furthermore, there was no significant difference in the heavy metals concentrations in the final effluent from the Phuthaditjhaba WWTP when compared to the effluent from the Harrismith WWTP. This thus indicate that these treatment plants discharge an effluent with heavy metals concentrations that may lead to ecosystem harm and affect the quality of the receiving streams.

TH044
Long-term monitoring and trend analysis of cyclic volatile methylsiloxanes (cVMS) in aquatic environments impacted by wastewater effluent
J. Kim, The Dow Chemical Company / Toxicology and Environmental Research and Consulting; J.A. Durham, Dow Corning Corporation / Toxicology and Environmental Research Consulting; R.M. Seston, Hyla Environmental Consulting, LLC / Toxicology, Environmental Research & Consulting; C. Mund, T. Böhmer, Evonik Nutrition & Care GmbH; N. Meguriya, Shin-Etsu
Cyclic volatile methylsiloxanes (cVMS) are widely used in industrial, personal care, and household applications. Because wastewater represents the major post-use disposal route for cVMS, the Silicone Industry Associations in North America, Europe, and Japan initiated a global monitoring program on major cVMS species in surface sediment and aquatic biota: octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6). Aquatic environments that were selected as study areas for the monitoring program were impacted by municipal wastewater effluents and included Lake Pepin (USA), Lake Ontario (on the border between Canada and the USA), Oslofjord (Norway), and Tokyo Bay (Japan). The target objective of the monitoring program was to determine if cVMS concentrations were stable or changing, based on detecting with 80% power (β=0.20) a statistically significant (α=0.05) annual rate of change of ±6% per year (net change of -27% to 34%). Target matrices for each study area include surface sediments, a high trophic level piscivorous fish, and several low trophic level forage fish and benthic invertebrates near the base of the food chain of the predator. The first project period was from 2011 and 2016. Generally concentrations of cVMS in biota and sediment showed decreasing trends over the period, but the statistical power was relatively weak in many cases. Thus considering the yearly variations, concentrations of cVMS in biota and sediment have been stable or decreasing in most monitoring locations based on the statistical criteria aforementioned. This presentation will discuss more details of monitoring data and their trends over the project period by location and species.

TH045
Distribution of rare earth element concentrations in leachate originating from electronic waste disposal
M. Makombe, V.S. Somerset, CPUT / Chemistry
Rare Earth Elements (REEs) form critical elements required in technological accessories. Their presence in electronic waste gives leads to environmental pollution. Therefore, there is a constant necessity for accurate data and reliable fast analytical methods. This review discusses and compares the methods given for the determination of rare earth elements and heavy metals in electronic waste and other associated environments. Classical analytical methods such as mineral acid dissolution and alkaline fusion are commonly employed. Various instrumental techniques that have been used recently includes electrothermal vaporiser or laser ablation accessory connected to ICP-MS or ICP-OES, X-ray Fluorescence and electro-analytical techniques. These methods of analysis including their accuracy, limit of detection (LOD) and limit of quantification (LOQ) are compared and discussed. These methods of analysis including their accuracy, limit of detection (LOD) and limit of quantification (LOQ) are compared and discussed. This study further investigates the preparation, dissolution methods and instrumental techniques with a focus on rare earth elements in electronic waste and its disposal into the environment.

TH046
Metals and Radionuclides: assessment of molecular effects of combined exposures using HepG2 cells as biological model
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The uranium mining legacy in Portugal (and in other parts of the world) had a significant impact in the environment and in human populations in the nearby areas. The residues produced are rich in metals and radionuclides and thus are a source of exposure to low levels of ionizing radiation. In addition, exposure to metals like zinc, manganese, iron, nickel etc, besides uranium also occur. In order to evaluate the genotoxic effects in human cells when exposed to a mixture of metals and low dose ionizing radiation, we exposed HEPG2 cells at different concentrations of a uranium mine effluent, for 96 hours. The time and concentrations of exposure were selected considering the concentration gradient of a uranium mine effluent discharge along a stream and water runoff from the mine ponds. Preliminary tests were performed to ensure that only sublethal damage was induced to the cells. Comet assay was employed to assess DNA damageand qPCR

was used to determine alterations in the gene expression of selected tumour suppressor genes and oncogenes. The comet assay results showed that effluent concentrations higher than 5% induce significant loss of DNA integrity and that DNA damage increased at higher concentrations. As for the gene expression analysis, results showed a significant under-expression of the tumour suppressors genes *TP53, ATM*and the fibroblastic growth factor *FGF2*and a significant overexpression of growth arrest and DNA-damage-inducible*GADD45A*gene. The data gathered demonstrates that the exposure to metals and radionuclides simultaneously promoted genotoxic effects in HEPG2 cells. As with other studies, our results reinforce the idea of a negative impact of a multi-stress environment such as uranium mines on the environment, more specifically on animals and humans living in the immediate vicinity. Keywords: Metals, radionuclides, DNA damage, gene expression alterations

TH047

Supporting water management strategies in mining using ecological risk assessment (MSc Thesis)

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The aim of this research is to assess the usability of ecological risk assessment for improving water management strategies in gold mining. The case study of ecological risk assessment of Kittilä gold mine is carried out to discover the possible ecological impacts of the mine discharge waters on the receiving rivers as well as to possibly predict the future impacts on the rivers after the mine expansion is conducted and new permit regulation limits for water discharge are set. The data applied in this study includes both monitoring data from the Kittilä gold mine (Agnico Eagle Finland Oy) and data gained through sampling. The environmental sampling conducted for this study included water, benthic fauna and sediment samples. The samples were taken from Seurujoki River, upstream and downstream from the mining site, and Loukinen River, downstream from the mining site. The concentrations of elements in the waters of the studied rivers were compared to national and international guidelines and Predicted No Effect Concentrations (PNECs), which are derived using and the Species Sensitivity Distributions drawn according to data from US EPA Ecotox database. The results suggest that the slight decrease in benthos diversity in Seurujoki and Loukinen Rivers could be caused by the mine waste water effluent, the ecological state of them still remaining mostly good. The impacts on water and sediment quality are quite local. There was indication of most significant elements of the mine discharge being SO4, Sb and Ni considering potential ecological risk. Ecological risk assessment tools appear to be useful in supporting gold mine water management strategies. It helps in utilization of the monitoring data: i.e. allocating the load to the possible effects and operations to improve water treatment and management. However, implementing site-specific guideline concentrations for example by the use of SSD would require more toxicity data.

TH048

Lessons from a multi-biomarker study in rivers: insight into challenges for biomonitoring

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In ecotoxicology, biomarkers are common tools that help to detect exposure or effect of substances on organisms. They are now used as part of regulatory monitoring on marine environment (Marine Strategy Framework Directive). In French marine ecosystems, for instance, the 2012-17-12 Decree on the definition of good ecological status of marine waters requires the measurement of 5 biomarkers on bivalves and/or fishes. On the other hand, in freshwater ecosystems, the Water Framework Directive (WFD) does not currently require biomarkers measurement, and only refers to them as a method that can be used to complement an environmental monitoring strategy. Questions remain about the interpretation of biomarker responses in freshwater ecosystems. This study aims to assess the spatiotemporal variability of biomarkers response when applied to freshwater streams. To achieve so, a set of 7 biomarkers was measured on European chub (*Squalius cephalus*) in September, 2016 during the environmental monitoring of the fish compartment in French rivers; fish were caught on streams where EDF (*Électricité de France*) electricity production sites are located. From molecular to individual scale, genotoxicity (comet assay, DNA adduct, micronucleus), neurotoxicity (AChE activity), and immunotoxicity (lysosomal integrity), as well as endocrine disruption (vitellogenin dosage), biotransformation (EROD activity) and development indices were accounted for. These biomarkers were selected in accordance with the European Technical Report on Aquatic Effect-Based Monitoring Tools (2014). This work provides insight into challenges for biomonitoring, and especially into the determination of reference values (or control values) and on the natural biological variation that can make the interpretation challenging. A focus is also made on the interpretation of the biomarker responses with an integrative index, which enables the processing of a set of biomarkers responses into one single score: the Integrated Biomarker Response (IBR). The pros and cons of two IBR versions will be weighed up to offer a critical review of this methodology when it comes to ecotoxicological assessment.

TH049

Complex responses of a biofilm to sewage outflow: a mesocosm approach

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Wastewater effluents represent the primary source of anthropogenic chemicals such as pharmaceuticals, personal care products, metals, and nutrients into the environment. Since they are not completely removed during the conventional wastewater treatment processes, they may pose a potential risk to the freshwater ecosystems. Whereas nutrients at certain concentrations may have a subsidy effect on biofilms, organic pollutants may have a stress effect. Thus, their combination can bring unexpected and contrasting effects in rivers. Of particular importance is to understand and assess how biofilms respond to chemical pollution and subsequently recover. Owing to the number of uncontrollable factors acting on rivers a way to evaluate the effect of wastewater effluent on natural systems is to study it under controlled and simplified conditions in a mesocosm scale experiment. We exposed twenty-four artificial streams to a range of WWTP effluent concentrations, from no effluent to pure effluent, during 34 days, followed by 22 days of recovery under no effluent conditions. Pollution load associated to the WWTP effluent was characterized analyzing physical-chemical parameters (pH, dissolved oxygen, and conductivity), nutrients, organic matter, heavy metals and personal care products and pharmaceuticals (i.e. ß-blockers, antibiotics, psychiatric drugs, anti-inflammatories…) as representatives of organic microcontaminants pollution in the water phase. The effects of chemicals on the biofilms were measured by means of structural (variations of biomass) and functional variables (such as the photosynthetic efficiency or the extracellular enzymatic activities). The overall observed response of biofilms to the wastewater gradient was fast and nonlinear in most of the analysed variables.

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TH050

Tiered framework for environmental risk assessment of industrial wastewater effluent discharges

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Industrial wastewater effluent discharges are undergoing increasing scrutiny and present challenges for environmental risk assessment due to complex and dynamic composition. Although tools and methods for assessing effluents and their potential hazardous properties have improved, the sampling and laboratory testing of effluent discharges is limited by logistical issues related to often remote and not easily accessible locations. In this project, a solution to this challenge was evaluated through the application of a tiered framework to quickly and effectively prioritise wastewater effluents for which further investigations are required. Discharges from a set of industrial sites with different production profiles as well as distinct wastewater treatment facilities were investigated. A set of screening methods was applied as the first tier in the assessment framework to provide information on the inherent hazardous properties of the effluent (persistence, bioaccumulation and toxicity). This toolbox consisted of: (i) the *in vitro* Microtox toxicity test, which measures toxicity in terms of changes in the bioluminescence of bacteria; (ii) a biomimetic solid phase microextraction coupled with gas chromatography (SPME-GC), to assess potentially bioaccumulative substances; (iii) target chemical analysis, to quantify a list of relevant compounds according to national and international (e.g. OSPAR) regulations; and (iv) Quantitative Structure Activity Modelling (QSAR), to estimate the toxicity of the identified components to aquatic organisms. In addition, the effects of aging on the toxicity and SPME-GC profiles was investigated through an experimental setup. Preliminary results indicate that the Microtox, SPME-GC and chemical analysis profiles vary considerably between the different industrial sites. Furthermore, after aging of samples, not always the reduction in the SPME-GC total area was correlated with a reduction in the toxic effects. Currently, wastewater effluents and respective sites are being evaluated regarding on the need to proceed to higher tier investigations, which can include Whole Effluent Toxicity testing and 3D-dispersion models. As an outcome of this project, it is foreseen that environmental risk assessment will be improved by the incorporation of effluent-specific science-based criteria, supporting decision-making in the context of local and international regulations, and ultimately promoting the protection of human and environmental health.

TH051

Effect of Differing Regulatory Guidance on Predicted No Effect

Concentrations (PNECS) for Active Pharmaceutical Ingredients in Industrial Wastewater Discharges

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Wastewater discharge permits may not specifically regulate chemicals that are unique to the pharmaceutical and animal healthcare industries, such as active pharmaceutical ingredients (APIs). Release of these chemicals has the potential to adversely affect the environment in areas downstream of the discharge. The potential for such effects can be assessed by developing predicted no effect concentrations (PNECs) for APIs and applying them to the downstream locations where exposures could potentially occur. The absence of a globally harmonized approach for the derivation and application PNECs can lead to substantial differences in the conclusions of risk assessments. To explore this issue, PNECs for aquatic organisms, wildlife, and humans were calculated for a variety of APIs spanning several therapeutic classes using two approaches: U.S. EPA's Great Lakes Initiative (40 CFR 132) and the European Union's Water Frame Work Directive (2000/60/EC) methods for deriving water quality standards. PNECs were calculated for several receptor-exposure scenario combinations, including acute and chronic exposure for aquatic organisms, chronic exposure for wildlife, and chronic exposure for humans associated with drinking water and recreational exposure scenarios. The results were variable within a therapeutic class, with PNECs that varied by 2,500-fold on average across both calculation methods and the previously described receptor-exposure scenarios. A sensitivity analysis was performed to evaluate the influence of the input variables and uncertainty in the underlying datasets on the calculated acceptable concentrations. Additionally, PNECs calculated using principles of the VICH's GL36(R) guideline for establishing microbiological acceptable daily intakes for antibiotics are compared with antibiotic resistance-based PNECs that have been published in the literature for several APIs. The effect of using US and EU-based approaches for evaluating the dilution capacity of receiving waters on the calculation of acceptable daily water discharges will also be discussed.

TH052

Biofilm communities as indicators of ecological impacts caused by treated and untreated abandoned mine effluents

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Mining activities have been performed since the Bronze Age and produce huge amounts of waste because the ore is a small fraction of the total volume of the mined material. Wastes produced by mining may reach surface waters both directly by effluent discharge and/or indirectly by seepage through embankments. About the 70% of the metal and salt mines in EU countries are classified as "abandoned" and represent an uncontrolled source of chronic pollution to receiving freshwater bodies because of the lack of any regulation about wastewaters treatment. The specific hazards posed by chemical stressors harboured in mine effluents to the aquatic environment mainly depend on its fate which influence its bioavailability and ecotoxicity. Biofilms are complex microbialcommunities with a relevant role in aquatic ecosystem functioning that are considered as effective bioindicator of ecological impacts and are recognized as necessary target within the Water Framework Directive. This study aims to evaluate the efficiency of an innovative treatment technology based on membranes and electrocoagulation (developed in the framework of the Life DEMINE project) for the reduction of the ecological impacts generated by metals and salts abandoned mines. Twodifferent experiments have been carried out under controlled conditions in microcosms to evaluate the ecological impacts of a) metal and b) salt mine effluents treated with different technologies using natural biofilm communities as bioindicators. Specifically we compared the behavior of control biofilm communities with the responses observed after a two weeks addition of water coming from mine effluents untreated (U) and treated with membranes (M), Electrocoagulation (E), Electrocoagulation after membranes (F) and a mix of water coming from M and F that would be the final effluent (EF) of the innovative technology developed. Untreted mine effluents (U) significanlty affected river biofilms by reducing its photosynthetic efficiency and phosphorus uptake rates whereas treated effluents barely provoked biological responses that, when observed, were generally recovered. Finally, the metal mine untreated effluent caused significant changes of the phototrophic biofilm compartment which shifted from diatom to green alge dominated community. This study demonstrated that the innovative technology developed in the Life DEMINE project significantly reduces the ecological impacts caused by abandoned mine effluents on biofilms.

TH053

The Omega-3 Index of macrophytes to improve the assessment of the treatment performance of constructed wetlands receiving treated wastewater

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Effluents from wastewater treatment plants (WWTP) contain chemicals that are not removed (metals, pharmaceuticals, hormones...) and are therefore considered as a source of pollution for the aquatic environment. Constructed wetlands receiving treated wastewater (CWtw), built between the WWTP and the receiving environment, can help to limit the impact of WWTP discharge on the natural environment. Currently, the methods used to evaluate the treatment performance of these processes are mainly based on physicochemical parameters. However, the wide variety of contaminants that can be encountered and their variable concentrations over time (sometimes below the limit of detection of equipment) constitute a limit of the use of such tools. Moreover, these methods do not provide information on the toxic impact of the treated water on the recipient ecosystem that is one expectation of the WFD. Biological tools that provide a global response to toxic impact of contaminants on the ecosystem can fill this gap. The Omega-3 Index, a standardized biological tool that has been proven it worth to assess soil quality (ISO/CID 21479), could be also used to assess water quality. In this context, the Omega-3 Index of reed leaves was tested as a biological tool to assess the treatment performance of CWtw and, as a consequence, the improvement of water quality. This biological tool was implemented on the experimental sites of the MARGUERITTES and BIOTRYTIS projects located in the suburb of Nimes and Bordeaux Métropole. These projects aimed at studying the functioning and the real efficiency for the removal of major pollutants and micropollutants of CWtw with different configurations (ponds, meadows, ditches built with soil and adsorbing materials). The Omega-3 index, measuring on reed leaves harvested upstream and downstream a pond-CWtw, highlighted that the reeds are more stressed upstream than downstream and therefore suggests that the upstream soil retain more phytotoxic compounds. It also showed that the reeds are more stressed on CWtw built with absorbing materials than those constructed with soil (meadows and ditches). Furthermore, the omega-3 index was found to be correlated to the water quality improvement determined by the classical biochemical parameters (BOD, TSS…) and some micropollutant contents. From a regulatory standpoint, this biological tool can help to better evaluate the treatment performance of CWtw and to assess the complex effects of pollutants.

TH054

PLEUROTUS OSTREATUS IN DEGRADATION OF SELECTED PHARMACEUTICALS: STUDY ON REMOVAL ABILITY, TOXICITY AND POSSIBLE METABOLITES

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Active pharmaceutical ingredients (API), present in effluents from wastewater treatment plants, constitute a serious problem discussed throughout the world [1]. When the effluents are introduced to the surface water, the API can exert a toxic effect both to the aquatic environment, biosystems basing on aquatic life and human [2]. In order to minimize the risk of the toxic effects, novel methods of the wastewater treatment should be applied. One of the method could be the application in the biological treatment of wastewater the organisms, that could remove API, like fungus from *Basidiomycota* [3]. The first aim of our study was to determine the ability of fungus from *Basidomycota* named as *Pleurotus ostreatus* to degrade selected API (n = 8). The second aim was to compare the toxicity of pre-cultivation and after-cultivation medium. At the last step we proposed metabolites as products of the degradation process carried out by *P.ostreatus*. The *P. ostreatus* cultures were carried out for five days in Sabouraud Dextrose Broth medium. API were quantified by validated LC-MS method. The toxicity was determined by bioassay based on *Spirostomum ambiguum* and *Vibrio fischeri.* **The metabolites were found using Compound Discoverer software. The obtained results showed that *P. ostreatus* is able to degrade majority of examined compounds. The toxicity of after-cultivation medium was lower than \n pre-cultivation medium. The detected metabolites were mostly products of oxygenation or demethylation reactions. To conclude, *P. ostreatus* can be useful in degradation of pharmaceuticals. Moreover, obtained results suggest its utility to wastewater treatment. <strong> References** Desbiolles F, Malleret L, Tiliacos C, Wong-Wah-Chung P, Laffont-Schwob I. 2018. *Occurrence and ecotoxicological assessment of pharmaceuticals: Is there a risk for the Mediterranean aquatic environment?*. Science of The Total Environment 639:1334-1348 Silva LJG, Lino CM, Meisel LM, Pena A. 2012. *Selective serotonin re-uptake inhibitors (SSRIs) in the aquatic environment: An ecopharmacovigilance approach.* Science of the Total Environment \n 437:185-195 Mir-Tutusaus JA, Baccar R, Caminal G, Sarrà M. 2018. *Can white-rot fungi be a real wastewater treatment alternative for organic micropollutants removal?*. Water Research 138:137-151

TH055

Purification with Moringa oleifera of a pretreated wastewater from the Chinampera Zone of México city

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Water is the fundamental element for the proper functioning of ecosystems in the "Chinampera" Zone which was cataloged as Natural and Cultural Heritage of Humanity by UNESCO since 1987. However, water is contaminated with pathogenic microorganisms, endocrine disruptors (including organochlorine pesticides), heavy metals and high salinity due to traditional agricultural activities. Therefore, the development and application of sustainable technologies is required to conserve and use the water resource in agricultural activities. In this project, the processes by which the contaminants can be removed from the seeds of *Moringa oleifera* and their effect on the water quality, in the improvement of a Xochimilco "apantle" will be investigated. The properties of the extract of *M. oleifera* seeds related to the removal of pathogenic microorganisms and endocrine disruptors were evaluated. *Escherichia coli* was isolated from wastewater of Xochimilco, and was considered as a model of pathogenic microorganisms, and *Pseudomonas putida* as a representative microorganism that degrades organic compounds; Lindane was used as a model for organochlorine pesticides. Based on the exploratory results, it was determined that the extract seeds presented characteristics of a surface agent, giving a positive emulsification index ($E_{24\%}$) and a decrease in surface tension (ΔTS). A critical micelle concentration (CMC) of 700 and 1500 ppm of the dehydrated seed extract was determined at 105°C and natural with a ΔTS= 18 and 20 mN/m and ($E_{24\%}$) of 30 and 17%, respectively. Both with 0.86 mm particle size of the seed. With these values a fractional 2^{4-1} factorial experimental design was established to evaluate the effect of the CMC, lysis time, *M. oleifera* particle size, contact time, salinity and pH in the inhibition of *E. coli* growth. Finally, seeds will be applied to a water pretreated in a biofilter in the "Chinampera" Zone, to improve the quality water used in irrigation.

TH056

The principle of the adverse outcome Pathway in the context of testing the antidepressant venlafaxine

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The worldwide production and use of pharmaceutical products has more than doubled over the last decade. A high interest could be found for the production of antidepressants whose neuroactive characteristics influence and change the functionality of different human transmitter systems. The discharge into the aquatic environment has also risen sharply due to the higher production rate of pharmaceuticals. An insufficient treatment of wastewater regarding the elimination of antidepressants and due to the higher production rate of neuroactive substances these drugs can get into the aquatic environment and cause neurotoxic effects like changes in the swimming behaviour of different fish species. One reason for comparable reactions in fish are the strong homologies of conserved genes which regulate basic functions of major systems like the serotonergic system. The analyses of pharmaceutical mixtures proves to be very difficult in practice because it is difficult to analyse synergistic and antagonistic effects especially if the environmental sample consists of many substances with different modes-of-action. A relatively new and auspicious model that can be used for the analysis of chemical mixtures with different modes-of-action is the principle of the adverse outcome pathway (AOP). The clarification of adverse effects such as changes in the swimming behaviour by analysing the underlying molecular initiating events represents a modular approach which has a great potential for the clarification of the primary key drivers. In order to validate the evaluation steps of pharmaceutical mixtures, the AOP evaluation was initially applied to zebrafish embryos exposed to the antidepressant venlafaxine. The results showed an increase in the swimming activity of 116 hpf old zebrafish embryos exposed with the antidepressant for 24 h. The analysis of the gene expression rate showed that especially genes of the serotonergic system had a log2 Fold-change rate, which was \n 2.5 times higher in comparison to the negative control. By including molecular pathway data, the ME could be traced back from the AO to the MIE and presented in the AOP concept. The results showed that the application of the AOP concept is possible for the analysis of pharmaceuticals and that it simplifies the analysis. An application to pharmaceutical mixtures is thus possible and will be performed in the future for a wide range of drugs within a network analysis.

TH057

The neuroactive cocktail: How to analyse the effects of the antidepressant clozapine in an AOP context

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Hospital wastewater is a mixture of different pharmaceuticals with diverse modes-of-action which show effects on different biological levels. Antidepressants are one sub-group of pharmaceuticals which are designed for the evoke of neuroactive changes in human and a worldwide used atypical antidepressant is clozapine. Strong similarities lead to comparable neuroactive effects in aquatic organisms which endangers the structure and stability of the ecosystem. One approach on how to structure and organize the effect analysis from an adverse outcome (e.g. behaviour) to the elucidation of molecular causes is the concept of the adverse outcome pathway (AOP). It works with a clear event chain between the molecular initiating event over different molecular events till the adverse outcome and it could also be used for the description and evaluation of complete adverse outcome networks. The swimming behaviour was tested with a DanioVision observation chamber in a light-dark rhythm with 10 min period length and a total testing time of 90 min. Zebrafish larvae treated for 116 h with clozapine showed an increase of the swimming activity of more than 40 % at a concentration of 12.5 μM in comparison to a negative control. Following the test protocol designed on the results of different pre-studies with pure substance investigations with clozapine and venlafaxine, the molecular modes-of-action were analysed with a real-time quantitative PCR (rt-qPCR) StepOnePlus system from ThermoFisher. Focussing on changes in the expression rate of genes related to the serotonergic system an increase of the Log2 Fold-Change ratio for *scl6a4a* and *htr5a* with more than 35 % for the highest exposure concentration of 12.5 μM in comparison to the negative control. The results show that the different components of the pharmaceutical mixture have a synergistic effect which provoke a hyperactivity reaction in zebrafish larvae. The behavioural reactions of zebrafish larvae against the exposure to different antidepressants show that the environmental risk assessment of this chemical is not sufficient to protect and to fulfil the aims of the Water Framework Directive 2000. That means that new approaches on how to test and analyse these substances is necessary and the AOP concept is an innovative way to solve the problems of future environmental risk assessment in the field of pharmaceutical testing.

TH058

Do scrubber water discharges from ships place additional stress on the marine environment?

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With the objective of significantly reducing negative environmental impacts of air pollution from international shipping and improving air quality in coastal regions, the use of low sulphur fuel is being gradually established by the International Maritime Organization (IMO). As of January 2015, marine fuel with a maximum sulphur content of 0.1% only is allowed in Sulphur Emission Control Areas (SECAs). To meet the oncoming requirements for lower emissions, shipping companies can also install exhaust gas cleaning systems (scrubbers) in which exhausts are washed with seawater and subsequently discharged into the sea. As a consequence hazardous substances such as heavy metals, polycyclic aromatic hydrocarbons (PAH) and other organic combustion products are likely to be released into the surrounding water, possibly at elevated concentrations which could have adverse effects on the marine environment and infringe environmental quality standards. Modeling studies suggest a containment of harmful effects due to the quick dilution of discharge water. However, sufficient toxicity data are missing to confirm these assumptions especially if scrubbers will be used at a larger scale in regions where dilution and buffering capacity of the water body is limited. We addressed this knowledge deficit by comparing the ecotoxicological potential of scrubber discharge water from two different scrubber technologies at several stages along the cleaning process in terms of their (1) acute toxic effects on marine algae and luminescent bacteria, (2) endocrine activities using yeast-based bioassays, (3) mutagenic profiles by means of specific tester strains in the Ames fluctuation assay. The efficiency of alternative treatments of scrubber discharge water can be evaluated based on the detected ecotoxicological effects. Bioassay results obtained by this study are complementing a chemical characterization of scrubber discharge and thus make an important contribution to the environmental impact assessment of scrubber discharge against the background of environmental quality criteria defined by the European Water Framework Directive and Marine Strategy Framework Directive.

TH059

Risks of anticoagulants to fish in effluent-laden streams

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Anticoagulant rodenticides are used worldwide to control commensal rodents for hygienic and public health reasons. Despite considerable acute toxicity of several anticoagulants to fish, very little is known about their distribution, fate, and exposure routes in the aquatic environment. However, recent findings of anticoagulant rodenticides in raw and treated wastewater, sewage sludge, estuarine sediments, suspended particulate matter, and liver tissue of fish in the low ng/L and ng/g range, respectively demonstrate that the aquatic environment experiences a greater risk of anticoagulant rodenticide exposure than previously thought. To investigate whether anticoagulant rodenticides enter streams via treated wastewater effluents after bait application in municipal sewer systems, we analyzed tissue samples of fish from 25 wastewater treatment plant polishing ponds in Bavaria, Germany by liquid chromatography – tandem mass spectrometry following a QuEChERS approach for extraction and clean-up. These polishing ponds are exclusively fed by municipal effluents and annually stocked with carp (*Cyprinus carpio*) for six months to enable monitoring of potential adverse effects and bioaccumulation of contaminants. In addition, operators and administrators in charge of municipal pest control at specific sampling sites were surveyed to retrospectively obtain information about rodent control in associated sewer systems shortly before or during the 2015 bioaccumulation period of carp in respective polishing ponds. Anticoagulant rodenticide residues were detected in carp liver at 12 out of 25 sampling sites in the low ng/g range. Six sites revealed hepatic residues of one or more second-generation anticoagulant rodenticide (i.e. brodifacoum, difenacoum, bromadiolone). 82% of surveyed sampling sites confirmed sewer baiting with anticoagulant rodenticides in 2015. Products containing brodifacoum, difenacoum, bromadiolone, or warfarin were exclusively used. Notably, hepatic residues matched the reported active ingredients used for sewer baiting at six sites. Although effluent-dominated systems such as polishing ponds represent worst-case exposure scenarios for fish, our results clearly indicate incomplete removal of anticoagulants during conventional wastewater treatment and confirm high bioaccumulation potential of second-generation anticoagulants. Our findings also reveal potential causative associations between sewer baiting and hepatic anticoagulant residues in fish.

TH060

Implications of temperature stresses on the fate of micropollutants during wastewater treatment

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Biological treatment plants (WWTPs) show high variability in (micro)pollutants (MPs) removal, which complicates their exposure and hence also risk assessment. A lack of experimental data is limiting our understanding of how operational and environmental parameters, such as temperature, influence the potential and rates of MP biotransformation in WWTPs, and prevent the validation of exhisting modelling approaches. In particular, current environmental exposure assessment relies on Arrhenius-based models to estimate the biotransformation rates in biological systems at different temperatures, despite they neglect potential temperature-induced compositional and functional variation of the microbial community. This work investigates the predicting power of Arrhenius-based models of MP biotransformation rate in a biological system. Series of laboratory-scale batch studies simulated short (daily) and long-term (seasonal) temperature shifts (4-40°C range) in activated sludge, and investigated how these changes affect the biotransformation potential and rate to degrade a mixture of 93 environmentally relevant MPs. Experimental median biotransformation rate constants (k_{bio}) across all compounds showed a positive correlation of k_{bio} with temperature in the long-term studies, approaching an Arrhenius-like behaviour, while this correlation was lost above 20°C in temperature-shocked biological systems. However, assessment of individual MPs revealed unsystematic temperature responses that did not correspond in the short- and long-term studies. Under temperature shocks, similar temperature responses were found in MPs that undergo similar initial biotransformation reactions (substitution vs oxidative-type), while these reaction-specific temperature responses were mostly lost in the long-term studies, with the exception of a small group of compounds undergoing a conjugation-type reactions (i.e. mostly sulfonamides). 16S rRNA amplicon sequencing showed that temperature changes strongly affect not only the enzymatic activity (ribosomal RNA) of microbial systems, but also induce substantial alterations in community composition (DNA), even when the temperature fluctuations occur over short periods. These findings question the predictive power of the Arrhenius-like approaches in environmental modeling, and emphasise the need to more accurately evaluate the biotransformation potential of biological systems to ensure accuracy in chemical risk assessment.

TH061

EVALUATION OF THE ADEQUACY OF NUMERIC VALUES DEFINED IN THE TABLES OF BRAZILIAN QUALITY WATER LAW FROM THE TECHNICAL AND SCIENTIFIC POINT OF VIEW, TO ENSURE THE PRESERVATION OF ECOSYSTEMS

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For Lobo and Callegaro (2000), monitoring the quality of a water body includes physical, chemical and biological monitoring, it is important in two ways. According to Knie and Lopes (2004), in ecotoxicology the use of bioindicators allows the detection of substances at concentrations below the limits of detection by chemical analysis methods, since living beings respond in an integrated way to all disturbing agents, including produced effects by new substances in water, formed through water and effluent interactions. Thus, as an alternative complementation of the physical and chemical characterization of effluents, toxicological evaluation is recommended. In this study, acute effects (lethality and immobility) and chronic effects (change in growth, reproduction and survival) are evaluated in ecotoxicological tests using concentrations value provided for in Brazilian law. What helps in decision making for the formulation of public policies. In order to evaluate the behavior of organisms against chemical compounds of petrochemical, agricultural and pulp and paper industries, some compounds were chosen according to the abundance/frequency in their use to trace a comprehensive behavior of the organisms against such evictions resulting on main objective: "Evaluate the adequacy and pertinence of the numerical values defined in the tables of Resolution 357/05 of CONAMA from the technical and scientific point of view, to guarantee the preservation of the diverse ecosystems." Six substances were to chosen to be tested: Benzene, Toluene, Glyphosate, Atrazine, Aluminum and Phenols. The results obtained didn't show toxicity for the tested concentrations for some organisms and chemicals. When tested toluene and benzene there was no morphological changes or deaths in *Daphnia magna*, but with *Danio rerio* it is possible to see many morphological changes and unviable eggs. The tests with atrazine showed inhibition on the algal growth and morphological changes on the fishes, but no death on the acute test with Daphnia magna. For glyphosate, there was no changes in the algae growth nor mortality in the acute test with Daphnia magna, but there was signifies morphological changes in the Danio rerio fingerlings. First, those preliminary results show that we cannot only use one organism to test and create public policies on environmental protection. Second, in a general way, the Brazilian law for water quality seems to be adequate to protect aquatic ecosystem, whit some exceptions.

State of the Science on Emerging and Novel Poly- and Perfluoroalkyl Substances (PFASs) (P)

TH062

An outdoor aging study in Australia to investigate the release of per- and polyfluoroalkyl substances (PFASs) from functional rain jackets

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Waterproof and breathable textiles provide protection against bad weather conditions. 88 million outdoor jackets are sold alone in Germany per year which comprises approximately 40 000 soccer fields of exposed surface area (presuming that an average jacket has $3m^2$ textile weave). A large proportion of these textiles are treated with durable water repellent (DWR) impregnations that are based on side-chain-fluorinated polymers (SFPs) and are subject to several material stressors during their service life, such as variable weather conditions, washing and abrasion. These stressors facilitate the release of per- and polyfluoroalkyl substances (PFASs) from the surface weaves of functional textiles. The loss processes of PFASs from polymeric SFPs are complex and various mechanisms lead to losses, namely; the loss of unbound residuals, polymer degradation (i.e. cleavage of side chains) or material loss in the form of particles or fibre fragments. The loss processes are likely to occur simultaneously during the textile's use. Different PFASs, especially perfluoroalkyl acids (PFAAs), have been identified in textiles in previous research, but a lot of precursors may remain unidentified. The complex chemistry makes the quantification of the total release potential for PFAAs from outdoor clothing a challenging problem. To determine the emission potential for PFASs, samples of outdoor fabrics treated with SFPs have been exposed in a real weathering test on the roof of a house in Australia for 6 months. In addition, we applied abrasion and washing to some of the fabrics after weathering to simulate their lifetime use. As well as testing the technical performance (e.g. liquid repellency) before and after ageing, we have measured the loss of total fluorine (using combustion ion chromatography, CIC) that occurred during aging of the textiles. We also used targeted analysis to measure residual PFAAs in the aged textiles using liquid chromatography tandem mass spectrometry (LC-MS/MS). These analytical measurements were complemented with microscopic investigation of the fibre surface defects after aging using scanning electron microscopy (SEM). The experiments allowed a better estimation of the extent to which outdoor garments contribute to the emissions of PFASs and provided new insights into the loss mechanisms.

TH063

Analysis of novel fluorinated compounds in wastewater, South Korea

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Perfluoroalkyl substances (PFASs) have been widely used for fire retardants, surfactants, circuit, and fabric etc. for a long time due to their stable physicochemical properties. However, due to their toxicity and persistence, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and its salts were regulated on production and use by Stockholm convention and EU REACH since 2009. Therefore, F53B, ADONA, and Gen-X (also known as HFPO-DA) have been introduced as several alternatives to current used PFASs and their occurrence in water was reported by several researchers (Pan et al., 2018; Strynar et al., 2015; Sun et al., 2016). However, only a few studies developed and validated the new PFASs analysis method. Moreover, lots of studies have been focused on investigating even-number carbon perfluorinated sulfonates (PFSAs) and perfluorinated carboxylic acids (PFCAs) in environmental media and limited studies are conducted on odd-numbered PFSAs such as PFPeS, PFHpS, and PFNS. Therefore, in this study, we validated the extraction method for analyzing 30 fluorinated compounds including legacy PFASs, novel fluorinated compounds, and some precursors of PFSAs in water samples. Furthermore, to investigate the usage and contamination of fluorinated compounds in industrial fields of South Korea, we analyzed these compounds in 16 industrial wastewater samples collected from industrial complex located in Gumi, South Korea. The water samples were pretreated using solid-phase extraction (SPE) with WAX cartridge (150mg, 6cm^3, Waters, Milford, MA, USA) and analyzed using high-performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) in multiple reaction mode (MRM). The obtained accuracy of 30 PFASs ranged from 62.7 to 111.3% and the precision ranged from 1.60 to 9.51%, which were satisfactory on US EPA method 537 although one of compounds had below 70% accuracy. In addition, we investigated the instrument detection limits (IDLs) and method detection limits (MDLs) by using the optimizing method. The detailed results will be presented in the poster.

TH064

Changes in PFAS distribution from hot spots sources to the abiotic and biotic environment

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The environmental PFAS distribution in proximity to hot spots is not well investigated. Most studies have focused on concentrations of perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and related compounds. The distribution of other PFAS present in the technical mixtures, their distribution in the abiotic environment and selective accumulation in organisms at several trophic levels, needs to be investigated further. The PFAS mixture used at a hot spot source zone varies depending on desired properties for the intended application. As an example, the PFOS precursor N-EtFOSE (n-ethylperfluorooctanesulphonamidoethanol) is associated with production of water repellent paper. Thus, the environmental mixture near a factory producing water repellent paper, or downstream a wastewater treatment plant (WWTP), is expected to be different from the mixture near a fire fighting training area. We are carrying out investigations of PFAS distribution in the abiotic environment and the food chain near different point sources in Norway: 1) Bodø Airport in the north of Norway, and 2) Longyearbyen Airport on Svalbard in the high arctic – two airports were AFFF related PFAS are released to marine fjords. Site 3), Tyrifjorden, is a large freshwater lake in the southern part of Norway were high levels of PFAS have been observed in fish (perch livers). The major active sources of PFAS-contamination in the lake are considered to be residues of historical use, and the largest sources identified are the sediments outside a closed-down industrial area for disposable paper products (plates, bowls, cups, etc.), and a fire station. We will present a comparison of changes in distribution and concentrations of PFAS mixtures in water, sediments and aquatic biota at these locations. Our work has special focus on source specific profiles and the changes that occur in the environment resulting in species-specific profile differences. Uptake from water and sediments will be compared by investigating PFAS profiles in benthic versus pelagic species. We will discuss our approaches to environmental monitoring of PFAS in different environmental compartments, especially with respect to sampling of biota (choice of species and tissues). Results from our work will be a significant contribution to risk assessment and management, and for regulation and policy making for contamination from point sources with complex PFAS mixtures.

TH065

Decision analysis of risk management options in dossiers to restrict dangerous substances in REACH legislation

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Under the European Chemicals legislation REACH, 24 restriction dossiers have been adopted for reducing or eliminating the production, manufacture and use of harmful substances to protect human health and the environment from unacceptable risks posed by chemicals. In a restriction proposal, the dossier submitter (DS) proposes a risk management option (RMO), which is evaluated according to defined criteria (risk reduction capacity (rrc), proportionality, implementability, enforceability, manageability, monitorability) and compared to a baseline (business-as-usual situation). In addition, possible alternative RMOs can be compared to the baseline. Limiting, substituting or phasing out the production, manufacture and use of dangerous chemicals has substantial and potentially long-term implications for producers, consumers, and the environment. To better understand how decisions of restrictions are adopted, this paper analyses the determinants for selecting the RMO that was ultimately proposed. Specifically, we investigate whether or not the selection of the preferred RMO is objective, transparent, and comprehensible given the results of the criteria-based evaluation. We analysed all parts of the 24 dossiers addressing the evalution and selection of RMOs. Tools are for instance an explanatory evaluation, a criteria matrix, and/or a socio economic analysis (SEA). The latter two are proposed by the Eurpean chemicals agency. Eleven DS used the criteria matrix with the above mentioned criteria and 13 motivated their decision of the RMO in an explanatory discussion. However, the expected rrc was most decisive in all 24 dossiers for the final selection of the RMO. Eight DS used an SEA to compare the rrc and costs/benefits with the business as usual situation to find the best out of several RMOs. The trend is that the lower the costs and the higher rrc the higher the probability that an RMO is chosen. Hence, the majority of the dossiers did neither include a criteria based matrix nor an SEA, but based the selection of the RMO on qualitative reasoning only. To facilitate coherent and more objective decision-making on restrictions a harmonisation of the selection process of RMOs would be desirable. Furthermore, we suggest reducing the number of criteria, which would avoid obvious definition overlaps. Finally, our results imply that SEA does not (yet) contribute to a systematic and comprehensive comparison of costs (and benefits) and, hence, to the choice of an RMO.

TH066

Determination of plant uptake of modified polyfluorinated substances and their biodegradation products in soil and in plants

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After the EU-wide statutory restriction of the production and use of perfluorinated substances (e.g. Perfluorooctanoic acid [PFOA] or Perfluorooctanoic sulfonic acid [PFOS]) due to their proven tendency to be bioaccumulative and persistent organic pollutants in 2006, polyfluorinated alkyl substances (PFAS), which are not covered by the legal guidelines, replaced many of these compounds. One replacement strategy is the use of polyfluorinated substances based on a fluorotelomeric structure modified with functional groups as phosphates, sulfates, alcohols and acrylates or methacrylates. This investigation deals with different PFAS, namely 6:2 fluorotelomer alcohol (FTOH), 8:2 FTOH, 10:2 FTOH, 6:2 fluorotelomer sulfonate (FTS), 6:2 polyfluoroalkyl phosphate (PAP), 8:2 PAP, 6:2 fluorotelomer acrylate (FTAC), 8:2 FTAC, 6:2 fluorotelomer methacrylate (FTMAC) and 8:2 FTMAC. The substances were applied on different types of soil (approx. 0.2 - 1 mg/kg soil) which was planted with radishes (*Raphanus sativus).* The grown period of radishes was 60 days. In first experiments investigating the content of PFAS in soil samples by using a methanol extraction and LC-MS/MS analysis method, several polyfluorinated carbon acids [perfluoropentanoic acid (C5) to perfluorodecanoic acid (C10)] were found in concentrations of approximately 1 – 30 μg/kg soil. As none of these acids was applied on the testing soils, it can be postulated that biodegradation processes formed them during the growth period of the radishes. In addition to that, in the soil samples the applied substances (PAPs, FTOHs and 6:2 FTS) were not found. For the determination of the FTOHs, FTACs and FTMACs as well as possible biodegradation intermediates, a GC-MS/MS method will be designed in the following experimental steps. Furthermore, the grown radishes will be analyzed to ascertain the amount of PFAS and their degradation products as well as the resorption capacity from soil to the radishes.

TH067

Direct uptake and translocation of Perfluoroalkyl Acids (PFAAs) in hydroponically grown red chicory (radicchio)

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In the past decade, high concentrations of Perfluoroalkyl substances (PFASs) in the Veneto Region (northern Italy) have been detected, primarily in the groundwater. Due to the scarcity of research regarding the human health risks posed by PFASs, especially in this region, and the high importance of agriculture in Veneto, with food (particularly vegetables) intake being a major route of the human exposure to PFASs, a study with the final goal of assessing health risk for the Veneto population is needed. A perfluoroalkyl acids (PFAAs) uptake study was carried out with red chicory (*Cichorium intybus L., rubifolium* group) as the model crop, given its significance for the Veneto agriculture. *C. intybus* was grown in the hydroponic system in a greenhouse, where nutrient solutions were spiked with nine PFAAs, 7 perfluoroalkyl carboxylic acids (PFCAs), C4-C10, and

2 perfluoroalkyl sulfonic acids (PFSAs), C3 and C8, in three treatments with the nominal concentrations of each PFAAs respectively being 62,5 µg/L, 125 µg/L and 250 µg/L. Actual PFAAs concentrations in the nutrient solution were monitored weekly and the concentrations in the roots and leaves were separately measured at the end of growing cycle. Mass balances, root concentration factors (RCFs), leaves-to-root concentration factors (LRCF), leaves concentration factors (LCF) and transpiration stream concentration factors (TSCFs) were calculated to evaluate the bioaccumulation of various PFAAs and their transfer from the nutrient solution to the radicchio roots and leaves. Analyses of the PFAAs concentrations confirmed that the short-chained PFAAs (C3-C6) accumulate mostly in the vegetative plant compartments, the edible part of radicchio, while the long-chained ones (C8-C10) adsorb to or are taken up more by the roots, resulting in a concentration of PFBA (C4) higher than 2000 $ng/g_{w.w.}$ in the leaves and concentrations of PFDA (C10) and PFOS (C8) up to 20000 $ng/g_{w.w.}$ in the roots (250 ug/L nominal treatment). Plants from the two treatments with the highest PFAAs' concentrations manifested physiological changes (discoloration, inhibited roots and leaves growth), with the smaller improvements with the concentration decrease in the nutrient solutions over time. Trends of increasing root concentrations and RCFs with chain-length increase of the PFCAs were noticed, while the LCFs, even though showing a trend of the linear decrease with the chain-length increase, were higher than expected for the PFHpA (C7).

TH068

Emissions and Fate of Fluorotelomer Alcohols in Municipal Sewage Treatment Plants

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Volatilization of fluorotelomer alcohols (FTOHs) from municipal sewage treatment plants (STPs) and subsequent atmospheric transformation contribute to the occurrence of perfluorocarboxylic acids (PFCAs) in the urban atmosphere and even in remote background regions. To date, a detailed, mechanistic description of how, and to what extent, the volatized FTOHs influences the ambient environment is inadequate. To this end, we determined FTOH concentrations in wastewater (influents and effluents) and the overlying air in a representative STP in Beijing, calculated FTOH emission flux from water to air based on concentration gradient, and estimated the magnitude of atmospheric emissions. Our results indicate that, among all investigated FTOHs, only 6:2 and 8:2 FTOHs are detectable in air and wastewater, with the former dominant in the air (55-69%) and the latter in wastewater (64%-73%). Seasonal (temperature) difference is absent in FTOH concentrations in wastewater but in those in the air. Emission fluxes from aeration and sedimentation tanks to the air above are estimated to be 122-375 $ng \cdot m^{-2} \cdot d^{-1}$ and 5.2-78 $ng \cdot m^{-2} \cdot d^{-1}$, respectively, depending on the ambient temperature. Each year, STPs across Beijing are estimated to receive 15 kg FTOHs along with domestic sewage, and to release nearly half (7 kg) to the environment, 30% of which enter the atmosphere. This implies that each resident in Beijing releases 2.6 mg FTOHs to the atmosphere per day. This presentation illustrates the environmental fate of FTOHs in STPs and provides a reference of emission level for other megacities similar to Beijing.

TH069

Investigation of PFAS concentrations in US food products.

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Perfluoroalkyl and polyfluoroalkyl substances (PFASs), in particular perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have been widely studied due to their persistence, distribution, toxicity and bioaccumulation in humans and the environment. These compounds can enter the environment through landfills (products containing stain repellents, food packaging), wastewater treatment plants (industry waste), and from the use of firefighting foams (aqueous film-forming foams [AFFF]. The heavy use of PFAS compounds has led to their wide-spread presence in the environment.\n The PFASs have been found at relatively high concentrations in surface water, groundwater, and soil surrounding airports and military bases where AFFFs have a history of use. Recently several contamination events in the United States caused concerns of possible contamination of food commodities produced using contaminated waters. An QuECHERS LC-MS/MS method has been developed for PFASs in a number of commodities, including fruits, vegetables, milk, cheese, cattle silage and other foods. In this presentation, method development and validation and results of the analysis of these food products and non-implicated samples purchased on the US market will be discussed.

TH070

Investigation of the Feed to Egg Transfer of PFAS Including the Transformation of PFAA Precursors in Laying Hens

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Per- and polyfluorinated alkyl substances (PFAS) are widely used in industry and in a wide range of consumer products due to their outstanding properties. Among the PFAS, the group of perfluoroalkyl acids (PFAA), which can be divided into perfluorocarboxylic (PFCA) and perfluorosulfonic acids (PFSA) depending on the functional group, is best characterized. Several PFAS are known precursors for PFAA which, due to their chemical stability, are persistent, bioaccumulative and toxic. The distribution of PFAS is ubiquitous. They were detected in animal, water, plant and soil samples. Due to their detection in human samples and drinking water, investigations of PFAS and especially PFAA precursors also come into focus in terms of consumer protection. In a feeding study conducted by the German Federal Institute for Risk Assessment (BfR), chickens were fed PFAS-contaminated feed. The distribution of PFAA in the animals was investigated. The results of the transfer study showed that the animals excreted higher amounts of PFAA through the egg than was ingested with the feed. In investigations of the feed different precursor compounds could be identified. In addition, the eggs were investigated for their content of precursors. Chicken eggs play an important role in the human diet, therefore a valid analysis for this matrix is necessary. At the same time, the transfer of precursors from contaminated feed into eggs can be traced. For this purpose, a multimethod for the detection of more than 40 PFAS is adapted to the matrix chicken egg. Using the Total Oxidizable Precursor (TOP) approach, precursors are degraded to their corresponding PFCA. By comparing the PFCA concentrations before and after the oxidation, it is possible to draw conclusions about the amount of precursors, and thus non-detected PFAS. This will provide an overview of the overall PFAS burden of egg samples obtained in the chickens transfer study.

TH071

Multimedia modelling of the fate of per- and poly-fluoroalkyl substances in Fuxin, China

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We will report the current status and progress in the creation of a fugacity-based multimedia model for river ecosystems in urban and agricultural environments that surround two PFAS (perfluoroalkyl substance) point sources in Fuxin, China. The modelling work is being done using the experimental data from the work of Chen et al. (2018). The objective is to accurately describe the fate and transport of 25 PFASs in this environment. We will report and critically review predictions of physicochemical properties of many previously ill-characterized PFAS made by application of COSMO-RS solvation theory. Fugacity-based multimedia models will be created in the manner of Mackay (2001). Three models with increasing complexity will be created (Mackay Level I, Level II, and Level III). The quality of the model will be assessed by the degree of agreement between the predicted and experimentally measured PFAS levels in the various environmental compartments of the system. This research will shed light on the important processes that control the fate and distribution of these compounds and will provide a supportive framework for regulatory control and environmental remediation. Sensitivity analysis and uncertainty analysis will be performed on the estimates from each model, toward generating a model that can accurately predict concentration values of several PFASs in the urban and agricultural environments. The resulting models will also provide insight and understanding into the processes and important factors that govern PFAS fate in the environment. The primary sinks for PFASs will also be highlighted under the defined environmental conditions.

TH072

Perfluoroalkyl acids (PFAAs) in soil and groundwater: transport modeling

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PFAS have been reported in series of studies as a hazard to environment and human health. Mobility in aqueous (dissolved) phase and spread in aquatic systems are the main drivers for transport and distribution. Physicochemical properties of PFAS define a particular behavior for transition between different domains as from infiltration from surface water to advective transport in saturated media. Transport mechanisms, however, are subject to retention (retardation) factors that require better understanding. Transport and behavior of PFAS in saturated flow were studied for a site with contaminated groundwater in Ronneby Municipality, Sweden. The main objective was to reconstruct the contamination pattern and reproduce the temporal variation of PFAS in groundwater. Fieldwork was organized with support of local waterworks and municipality. The project was conducted as a part of epidemiological study on PFAS exposure and health effects. Environmental investigation included analysis of the media properties (soil/sediment), PFAS analysis and monitoring (surface and groundwater), and different sampling strategies. The transport/retention model was built on a detailed cross-section profile of the aquifer properties. The solute transport model was calibrated to estimate the pulse duration and retention time in soil media.

TH073

PFAS Terminology - Clear, Specific and Descriptive. How have we done?

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In 2011, a landmark publication, "*Perfluoroalkyl and Polyfluoroalkyl Substance in the Environment: Terminology, Classification and Origin*" (doi: 10.1002/ieam.258) set forth terminology and classification for per- and poly-fluoroalkyl substances (PFAS). PFAS are a diverse class of substances with vastly different physical, chemical and biological properties. In the paper, the authors strongly encouraged the global scientific community to adopt the terminology and classification presented and use clear, specific and descriptive terminology in writing. The authors specifically discouraged the use of broad, poorly defined terms and acronyms, like PFAS alone. How have we done? This paper will present an assessment of the published scientific literature for 2017 and 2018. A large number of the published papers have missed the mark by using the acronym PFAS to describe the work presented in the title and abstract when the work was really done on a subset of PFAS substances. Greater clarity, specificity and description would have been desirable. For example, many papers describe studies on perfluoroalkyl acids (PFAAs) not PFAS as an entire class. For a representative group of these papers, the use of terms that would be clear, specific and descriptive and thereby strengthen the work will be offered. In addition, papers which do hit the mark and provide clear, specific and descriptive titles and abstracts will be highlighted. The intent is to further engage the community to strengthen the commitment to more clearly communicate about PFAS and specific substances within it the broad class.

TH074

PFASs in a Tropical Estuarine Food Web: Trophic Magnification and Wildlife Exposure

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Biomagnification of per- and polyfluoroalkyl substances (PFASs) was investigated in biota from a tropical estuary in Bahia, Brazil. This area is one of the few regions in the world where a PFOS-precursor (the commercial pesticide Sulfluramid; N-ethyl perfluorooctane sulfonamide, EtFOSA) continues to be used under manufacture and use exemptions of the United Nations Stockholm Convention on Persistent Organic Pollutants. Samples of liver or muscle were obtained from bivalves (n=5), crustaceans (n=4), annelids (n=1) and fishes (n=12) covering 3 trophic levels (based on analysis of stable-nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes). ∑PFAS concentrations were dominated by PFOS, followed by perfluoroheptanoate (PFHpA) and perfluorotridecanoate (PFTrDA). Perfluorooctane sulfonamide (FOSA) and EtFOSA were also detected. Liver PFOS concentrations were highest in *Aspistor luniscutis* (45.1 ng g^{-1}) and lowest in *Genyatremus luteus* (0.23 ng g^{-1}). Evaluation of PFAS trophic magnification was achieved by converting liver or muscle-based concentrations to whole-body concentrations, which were then plotted against trophic level. To our knowledge, this is the first time PFAS trophic magnification has been investigated in Brazilian wildlife.

TH075

Poly- and perfluoroalkyl substances (PFASs) in global surface waters. A review of spatial and temporal trends

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Poly- and perfluoroalkyl substances (PFASs) are aliphatic organofluorine molecules with highly fluorinated carbon chain "tails," and a hydrophilic "head" groups of carboxylates, sulfonate, or phosphonates/phosphinates. Many are degradation products of more complex precursors. It has been well established that they are transported in wastewater and rivers to the oceans which serve as a terminal sink. This study builds on a review of data for PFOS in surface waters published up to early 2015 that was included in the Stockholm Convention 2nd Global Monitoring Report http://chm.pops.int/Implementation/GlobalMonitoringPlan/Overview/tabid/83/Default.aspx). Our goal was to provide an updated overview of spatial trends, and where possible, temporal trends of PFASs in oceans and freshwater globally, in order to assess effectiveness of national and global bans and provide a baseline for future work. In order to provide an estimate of the range of concentrations without including wastewater effluents we used results from the lakes, rivers and estuaries that included two or more sampling sites per river reach and identified by the study authors as being upstream or remote from urban waste water effluents. On large rivers, only data from mainstream river sampling sites were selected rather than tributaries. For marine environments we used results from open ocean cruises. We found that the spatial coverage of PFAS measurements in water was dominated by information for rivers in western Europe, Eastern China, Japan, and the Great Lakes in North America. For marine waters most data was from the North Sea, the Baltic, and the South China Sea while for open oceans measurements in the North Atlantic and the Arctic Ocean predominated. Results showed that ice and snow melt inputs of PFAS are likely to be important during spring melt events and need to be considered in timing of monitoring programs in polar areas. The presence of PFOS in remote sectors of the southern Ocean, the Arctic Ocean and the mid-North Atlantic, including in deep waters (>1000 m), illustrated the long range ocean transport of PFOS and other PFASs and their value as tracers of ocean circulation. Temporal trend information has improved over the past 5 years due to repeat sampling of some locations (eg in the Canadian arctic archipelago), as well as separate cruises especially for the Eastern North Atlantic, and the first evidence is emerging of declining PFOS concentrations.

TH076

Research Needs to Inform the Substitution of Polyfluorinated Substances New to Canada: Assessment of New Substances Subject to Regulation

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Substances new to Canada are subject to the New Substances Notification Regulations (NSNR) where prescribed information submitted by notifiers is assessed by the New Substances Program (NSP) of Health and Environment Canada. Per/polyfluorinated substances (PFAS) containing long-chain perfluoroalkyl groups were assessed by the NSP and found to be "CEPA toxic". These assessments were instrumental in banning long-chain perfluorocarboxylic acids (PFCAs) and their precursors in Canada (http://www.chemicalsubstanceschimiques.gc.ca/plan/approach-approche/pfca-apfc-eng.php). Further regulatory efforts to minimise exposures to these substances have led companies to propose "alternative substances" that are merely structural variants of those found to be of concern. "Informed Substitution" challenges researchers developing alternatives to proactively generate relevant information to delineate their potential health effects and environmental effects. Chronic hazard, metabolite data, multi-generational studies and monitoring of environmental media would facilitate comprehensive assessments but go beyond what is required under the NSNR. Such critical information would contribute to discerning substances which are viable PFAS alternatives from a health and ecological perspective. Assessment methodologies for new substances in general will be presented and those pertaining to polyfluorinated substances will be highlighted.

TH077

Short-chained PFAS in Finnish aquatic environment

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This study presents the results of PFAS-survey in Finland. Surface water and fish were sampled in locations with high to very low anthropogenic pressure including rivers, lakes and the Baltic Sea. Short-chained PFASs were detected in more than 60% of the river water samples. PFHpA was detected in 95% and PFHxA in 84% of the samples, and they were the most commonly detected substances after PFOA, which was detected in 96% of the samples. The concentrations were rather low, maximum concentrations being < 6 ng/L. PFBS and PFBA were not detected in the background sites. The concentrations were higher in rivers receiving effluents from municipal waste waters and there was a visible peak in concentrations during snowmelt period in spring. The riverine load of PFBA to the Baltic Sea was 17 kg, which even exceeds the loads of PFOS (10 kg) or PFOA (11 kg) of the same rivers in 2017. Short-chained PFAS have been measured from perch (*Perca fluviatilis*) and herring (*Clupea harengus membras*) tissue starting year 2014, and PFHxA has been detected once and 6:2 FTS in 3 samples. The other measured short-chained PFASs have not been detected in fish. As comparison, long-chained PFAS are commonly detected in the fish samples. The results show that short-chained PFASs are widely spread in the surface waters but do not accumulate in the fish tissue. They enter the waters via atmospheric deposition but also from point sources. Short chained PFASs contribute up to 54% of the total PFAS concentrations of the 23 studied PFAS in the rivers and their loading to the Baltic Sea even exceeds the load of PFOS and PFOA. These first results still leave us with many open questions regarding short-chained PFASs. The sources of these substances are not well known: we have little knowledge on PFAS-polluted soils and no data of atmospheric deposition of PFASs in Finland. The huge number of PFASs is a challenge and requires further development in the analytical methods, screening and monitoring activities as well as modelling the environmental fate of these substances.

TH078

Size-specific distribution of perfluoroalkyl substances (PFASs) in aerosols close to one of the major fluoropolymer manufacturing facilities in China

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Environmental Science and Analytical Chemistry (ACES)
The ubiquitous presence of per- and polyfluoroalkyl substances (PFASs) in the environment has attracted increasing public attention. The manufacture of fluoropolymers such as polytetrafluoroethylene (PTFE) is a major source of low-molecular-weight PFASs like perfluorooctanoic acid (PFOA). However, limited information is available in terms of direct emissions of PFASs into the atmosphere as well as the role of direct sources in the atmospheric transport of PFASs. In order to investigate the long-range atmospheric transport potential of directly emitted PFASs via airborne particles, size-segregated aerosol samples were collected in a residential area close to a fluoropolymer (FP) manufacturing plant in China. Ambient aerosol was sampled everyday over the course of 30 days using a 5-stage impactor (cutoff sizes were 12.2 µm, 4.05 µm, 1.35 µm, 0.45 µm and 0.15 µm) connected with a quartz fiber filter (for particles < 0.15 µm). The preliminary results of a pilot study demonstrated that a large portion (~40%) of perfluoroalkyl acids (PFAAs) was found in the < 1 µm size range. A sharp elevation in the concentrations of C6-C8 perfluorocarboxylic acids (PFCAs) was observed when there was wind coming from the direction of the FP plant, and the concentrations of individual PFCAs were significantly correlated with each other, indicating a common source. Among the PFAAs investigated, PFOA was the dominant one, and was about three orders of magnitude higher in concentration than the other PFAAs.

TH079

Zurich Statement on Future Actions on Per- and Polyfluoroalkyl Substances (PFASs)

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Per- and polyfluoroalkyl substances (PFASs) are man-made chemicals that contain at least one perfluoroalkyl moiety, $–C_nF_{2n}–$. To date, over 4,000 unique PFASs have been used in technical applications and consumer products, and some of them have been detected globally in human and wildlife biomonitoring studies. Because of their extraordinary persistence, human and environmental exposure to PFASs will be a long-term source of concern. Some PFASs such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) have been investigated extensively and thus regulated, but for many other PFASs, knowledge about their current uses and hazards is still very limited or missing entirely. To address this problem and prepare an action plan for the assessment and management of PFASs in the coming years, a group of more than 50 international scientists and regulators held a two-day workshop in November, 2017. The group identified both the respective needs of and common goals shared by the scientific and the policy communities, made recommendations for cooperative actions, and outlined how the science–policy interface regarding PFASs can be strengthened using new approaches for assessing and managing highly persistent chemicals such as PFASs. The Statement has been published in the open-access journal Environmental Health Perspectives, and this poster intends to present the Statement to a wider audience from academia, civil society, governmental agencies and industry.

Chemical Exposure via Plastics and Synthetic Rubbers: Quo Vadimus? (P)

TH080

Marine debris as a source of hazardous chemicals in the marine environment: Transfer of hexabromocyclododecane from expanded polystyrene debris to marine organisms inhabiting the pristine environment

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The role of plastic marine debris as a carrier of hazardous chemicals in the marine environment is an emerging issue. This study focused on expanded polystyrene (EPS, commonly known as Styrofoam) debris, which is a common marine debris item worldwide, and its additive chemical, hexabromocyclododecane (HBCD). To identifythe potential of EPS debris for the transfer and dispersion of chemicals to the pristine marine environment, two islands located in the southern part of Korea with very different EPS debris pollution levels (one contaminated islands and one non-contaminated island) were selected as study sites, and the exposure levels of HBCDs in marine organisms inhabiting each island were assessed and compared. Along with HBCDs, a typical sorbed chemical, polychlorinated biphenyl (PCB) were analyzed for comparison. No difference was observed in PCB levels in organisms such as oyster, limpet, and gastropods from two islands, while the HBCD concentration was significantly higher in organisms inhabiting contaminated islands than those inhabiting non-contaminated island (Mann-Whitney, $p< 0.05$). The result of this study indicates that EPS marine debris has a strong potential for source and carrier of plastic additives, HBCDs, in the marine environment.

TH081

Plasticizers in German rivers: DEHP and its emerging substitutes

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Plasticizers are among the most commonly used functional additives in PVC. Their proportion by weight in products can be up to 50 % w/w. The phthalate plasticizer Di(2-ethylhexyl) phthalate (DEHP) is a well-documented ubiquitous contaminant and frequently regarded as the predominant plasticizer in the environment. In the EU, the use of DEHP and other phthalates was banned because of their endocrine-disrupting activity. As a result, the production of these phthalates decreased significantly. Market data show that high-molecular-weight phthalates are now extensively substituting DEHP together with various types of non-phthalate plasticizers. However, the global plasticizers demand is continuously growing. The objective of this study was to obtain spatio-temporal trends for DEHP and its substitutes in freshwater systems. We analyzed suspended particulate matter samples (SPM) for the presence of 23 plasticizers, i.e. 17 phthalates and 6 non-phthalates. Samples from recent years were retrieved from the German environmental specimen bank (ESB), which covered 16 sampling sites from major rivers including Rhine, Elbe and Danube. In samples from today, we observed a higher diversity of softeners than in samples from the past together with a shift to less volatile substances. The high-molecular-weight plasticizer Diisononyl phthalate (DINP) is the most abundant plasticizer detected in the SPM-samples. Our results indicate a fast appearance of new plasticizers like Diisononyl cyclohexane-1,2-dicarboxylate (DINCH) in freshwater environments and we identified several compounds, for instance Di(2-propylheptyl) phthalate (DPHP), as potential chemicals of emerging concern with increasing levels. In addition to the substitutes, we continued to demonstrate DEHP in the major German rivers: this results in an increasing total exposure to plasticizers at some of the sites we investigated. Despite the strict EU regulations, DEHP levels have not been declining for some years, but are stagnating at relatively high concentration levels in some cases. This is in contrast to the decreasing levels of DEHP in human samples from the German ESB over the last decade.

TH082

Assessment of microplastics occurrence and composition in Swedish road waste water through a thermal degradation method

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The global production of thermoplastics has grown rapidly since the start of its large-scale production around the 50s. The different varieties of polymers produced have unique characteristics in terms of durability, production costs, weight, strength, flexibility and limited electric conductivity. Nowadays, the market of thermoplastics is dominated by four main classes of plastics, being polyethylene, polyethylene terephthalate; polypropylene, polyvinyl chloride, polymethacrylate, nylon, polycarbonate and polystyrene. Besides thermoplastics, rubber is also considered a class of plastic. Plastics are also used in road materials. To improve the properties of asphalt, polymers are added to some bitumen. The materials used are Styrene Butadiene and Styrene Ethylene Butylene Styrene copolymer. In brief, the polymers make the asphalt stiffer on warm summer days and more flexible on cold winter days. Another abrasion surface made of plastics on the roads is the road marking paint. This is made by mixing variable amounts of Styren-Isopren-Styren, Etylen-vinylacetat, Polyamide and acryl-monomer. City

dust in urban runoff is known as a significant contributor to the pollution of receiving waters. A preliminary study of microplastic abundance in Skagerrak, the coastal waters among Norway and Sweden, pointed out that a large fraction of particles found in the sea seems to be associated to city dust e.g. asphalt and car tires. To support this hypothesis four samples of road runoff close to the kerb on a road ending up into a stream located in a nature conservation area in Gothenburg were collected. Samples were characterized for plastic polymers and rubber abundance. The whole sample preparation, combining a sequential step of enzymatic and strong oxidizing treatments followed by density separation, was designed to avoid intermediate filtration steps, preserving the whole particle size range. Plastics and rubber content were estimated thorough a thermal degradation method based on a GCMS-pyrolysis technique adopting specific indicator ions for the detection of PP, PVC, PS, PMMA, PET, PC, NY, PE and rubber. The analysis of pyrograms showed that the sample preparation process was successful and resulted in optimal analytical conditions, leaving very limited matrix or background interference. PVC, PP, PVC PS PMMA, PET and rubber where quantified in the range of μg/L, with PVC and PP being the most abundant polymers. Rubber contents spanned from 1.7 to 7.7 μg/L.

TH083

Car tyre wear in freshwaters - a probabilistic mass balance modelling approach

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Microplastics have been detected in rivers and streams around the world. Car tyre wear is a major source of microplastics in the freshwater environment. Measurements of car tyre wear are scare, and when available they are fragmentary in both space and time. Moreover, the measurements cannot reveal all sources or transport characteristics, and therefore hamper the development of exposure assessments. Models can help to integrate these fragmentary data into more comprehensive exposure overviews needed for risk assessments. The models currently available are however focussing on small (sub) catchments or are on a very coarse scale. In this study we modelled the transport and fate of car tyre wear in the Netherlands. Based on the WFD Explorer 2.2., a national-scale, steady state mass balance model, the Dutch water network was divided into almost 19000 surface water units. We identified and quantified different point sources including over 300 waste water treatment plants and 9 cross-boundary flows, but also took into account diffuse surface runoff of tire wear particles. Emission input data were based on existing literature. We focussed on the variability and uncertainty of the particle properties and emission scenarios, using a probabilistic approach. Also, we calculated the export of car tyre wear to the North Sea. We found that car tyre wear concentrations vary several orders of magnitude between surface water units, both in the water and in the sediment. Large variations in car tyre wear concentrations were also found for specific locations and for the export to sea when varying the particle properties with the Monte Carlo approach. Our model provides the first comprehensive exposure assessment for car tyre wear on a national scale, and will form the basis for a prospective risk assessment, since modelled concentrations can be compared with effect thresholds for different aquatic organisms. Also, management measures can be included in the model, to illustrate how these measures would affect car tyre wear concentrations in freshwater systems.

TH084

Chemical additives in plastics: overview and possible prioritization strategies

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Plastics pervade every aspect of our modern life due to their cheap price and versatility associated with different chemical additives therein. These chemical additives may provide not only desired functionalities such as flame retardancy and plasticity, but also features such as color and smell that make the products more attractive to consumers. However, many additives are not chemically bound to plastics, and may be released during the production, use, disposal and recycling of plastics, leading to undesired human and environmental exposure to these chemicals and causing adverse effects in some cases. In contrast to the broad range of chemical additives that are currently used in plastics (e.g., it is recently estimated that over 900 chemicals additives have been used in different polypropylene (PP) products), the current research and risk assessment has focused on a limited number of chemicals such as heavy metals, phthalates and brominated flame retardants. One major cause of such phenomenon is that the identities of many chemical additives are yet unknown/unfamiliar within the wide scientific and regulatory community. This ongoing shortcoming has motivated us to develop a comprehensive database of chemical additives in plastics and to develop an adaptable prioritization strategy that would help to systematically focus resources on those most problematic substances, subject to protection goals. In the first step of this study, information on intentionally added additives in plastics was collected from publicly accessible databases, scientific literature, books, patents, and technical reports, including CAS numbers, chemical names, molecular structures, and different hazardous properties. The collected information was harmonized and compiled into one single database. In addition, predictive quantitative structure-activity relationship (QSAR) tools were used to fill in data gaps in physicochemical and toxicological properties. In the second step, a framework of adaptable prioritization strategies was developed based on hazard and exposure assessment and multi-criteria-decision-analysis. In particular, various selection criteria such as persistence, bioaccumulation, toxicity and release potential can be integrated and weighted to fit different purposes. To validate the framework, a case study on prioritization of substances that are critical to plastic recycling was conducted.

TH085

Evaluation of impacts of expanded polystyrene leachate on four marine microalgae

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Due to enormous amounts of plastic wastes in marine environment, the concerns about marine pollution and ecological impacts on marine organisms also have increased. Especially, plastic pollution which originated from expanded polystyrene (EPS) is serious in Korea. In this study, we used EPS leachate and four marine microalgae species to evaluate the impact of EPS on marine microfauna. Various EPS fragments and spheres with different size, shape, and concentrations were incubated with F/2 media for 28 days and four marine microalgae (*Dunaliella salina, Scenedesmus rubescens, Chlorella saccharophila,* and *Stichococcus bacillaris*) were exposed to these leachates for 10 days. After exposure, photosynthetic activities and cell morphologies were analyzed in each microalga. In results, cell growths of four microalgae were stimulated when they were exposed to high concentration of EPS leachate and the photosynthetic activities also increased. This result is assumed to be the effects of additive chemicals included in EPS and more future studies are needed. *Acknowledgement - This research was also supported by a research project titled, "Environmental Risk Assessment of Microplastics in the Marine Environment" from the Ministry of Ocean and Fisheries, Korea (PM60690).*

TH086

Microplastics as vectors of emerging contaminants in the terrestrial ecosystem: ecotoxicological impact

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We live in a world in which plastic wastes are accumulating in different ecosystems. For this reason, the study of the impact of plastics on the environment has become an **emerging area** of research. Recently, research on the environmental impact of plastics has acquired a new dimension through the discovery and study of **microplastics (MPs)**(plastic particles MPs have become an environmental problem because: (i) they are small enough to be **ingested by biota**, which affects their integrity and can accumulate in the food chain; and (ii) they can **adsorb contaminants** on their surfaces, serving as **contamination vectors**, especially organic compounds. MPs have been located in different systems (surface waters, oceans, sediments) and organisms, although their environmental impact has been studied almost exclusively in marine environments and coasts. However, there is an evident **lack** of MPs' distribution and impact data on the **terrestrial system.** The main sources of MPs in the terrestrial ecosystem are sludge from wastewater treatment plants (WWTP), urban runoff, agricultural mulching, as well as landfills and industrial areas. The MPs found in wastewater come mainly from the fragmentation of plastic waste, but also from personal care products and synthetic textile fibers. The presence of MPs in the terrestrial system can have harmful effects on different processes, not only their presence but their capacity to carry other contaminants, affecting indigenous organisms of the soil / water system. The MPs adsorb hydrophobic organic contaminants, since they have a high relation surface / volume. This makes them **vectors of contamination** with the consequent risk of dispersion of **emerging pollutants** that may exceed the WWTP. Therefore, a better understanding of ecotoxicological responses in vivo of MPs and their interaction with emerging pollutants should be considered a necessary and integral part of investigations into their impacts on the environment In this study, we have conducted a set of ecotoxicological bioassays: Microtox® Test, *Caenorhabditis elegans* Test, emergence and growth inhibition of two plant species (*Lactuca sativa* and *Zea* mays) to evaluate the potential ecotoxicological risks associated with three polietylene microspheres (blue, white and phosphoren blue) ranging 212-300 μm. In addition, we have tested the interaction of these MPs with the pharmaceutical ibuprofen, and the herbicide simazine. Keywords: emerging pollutans, bioassays, microplastics

TH087

Preliminary results of the urinary bisphenol A in adolescents

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Humans are exposed to bisphenol A (BPA) through wide range of everyday products such as metal cans, food and drink plastic containers, medical devices, toys, thermal papers etc. The widespread exposure to the estrogen-like compound, BPA is related to variety of health disorders including weight gain and insulin resistance, disrupted thyroidal function, cardiovascular adverse health effects and increased carcinogenic risk. However, the findings related to the BPA levels in adolescence and possible health effects are very scarce and limited. The aim of the study was to determine the prevalence of BPA in urine among adolescents as part of the survey about the association between human disorders and exposure to endocrine disruptors in Vojvodina, the north province of Serbia. The cross-sectional study was conducted on 30 healthy adolescents from Novi Sad, Serbia, aged from 10-21 years. After the enzymatic treatment the morning spot urine samples were preconcentrated using methyl tert-butyl ether as solvent for liquid-liquid extraction. BPA was analysed in samples by gas chromatography coupled with mass spectrometry using the deuterated bisphenol A as an internal standard. The urine samples from 17 adolescents (out of 30) were positive on BPA. The higher frequency of the detection was obtained in boys (75%) in comparison with girls (50%). The obtained results suggest that adolescents are ubiquitously exposed to BPA in this region. Therefore, the special attention should be paid on urinary BPA levels in adolescents, in order to evaluate the health risks that may be associated with the obtained high prevalence of BPA contamination (57%).
Acknowledgement - This research has been financially supported by the Provincial Secretariat for Science and Technological Development, AP Vojvodina, Republic of Serbia, Grant No 114-451-2216/2016.

TH088
Presence of different chemicals of concern in rubber crumb used in synthetic football fields
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A large volume of end-of-life tires (ELT) enters the waste stream in the world each year and recycled rubber from ELT is used as infill material in synthetic turf fields and playgrounds. After research published in the past 10 years, concerns exist regarding the safety of synthetic turf fields and the governmental agencies US-EPA, in April 2016 and European Chemicals Agency (ECHA) in February 2017 conducted independent studies, which have not provided consistent evidences and found the need to obtain further information, proving that this issue is not yet closed. Although ECHA has found no reason to advise people against playing sports on synthetic turf, it made specific recommendations as: inclusion in future possible changes in REACH legislation to decrease the maximum limit of 0,001% w/w of PAHs concentrations in the rubber granules, mandatory periodic measurement of PAHs levels in rubber granules by owners and operators, guidance of producers of rubber granules towards helping manufacturers and importers to test their infill material, adequate ventilation of indoor fields and users awareness of appropriated basic hygiene measures. While exposure assessment, toxicological studies and chemical characterization of toxic substances are still under study, technological measures to eradicate the substances of concern from the rubber granules and support the use of this material have not yet been considered. This work intends to shed light on the presence of different chemicals of concern (such as polycyclic aromatic hydrocarbons (PAHs), metals and other volatile and semi-volatile organic compounds (VOCs and SVOCs, respectively) in rubber crumb used in synthetic football fields, reviewing the scientific literature available so far in this subject.
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TH089
Proteomic and biochemical alterations in mussel gills after exposure to the organophosphate flame retardant TDCPP
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The organophosphate flame retardants (OPFRs) are a diverse group of substances used as flame retardants, plasticizer and anti-foaming agents in several consumer and industrial products including plastics. Like other additives, they are not chemically bound to the substrate material, and therefore they are released into the medium throughout the useful life of the products. This has resulted in the detection of the presence of OPFRs in air, soil, sediments and surface waters. Harmful effects of OPFRs have been detected in animals and humans. In the aquatic environment, the effects of the OPFRs reported are mainly in freshwater species. The aim of this study is to evaluate the effects of the OPFR tris (1, 3-dichloro-2-propyl) phosphate (TDCPP) on the gill proteome of the mussel *Mytilus galloprovincialis,* a sentinel species in marine pollution monitoring, as well as to assess the effects on molecular biomarkers, including a neurotoxic response as acetylcholinesterase (AChE) and a Phase II detoxification enzyme: glutathione S-transferase (GST). For that, mussels from a pristine area were exposed to the flame retardant TDCPP during 28 days, at a concentration of 10 μg/L. Gills proteomic analysis was performed by LC-MS/MS using "tandem mass tags" (TMTs) isobaric labelling. Stability of TDCPP in water and its bioaccumulation in *M. galloprovincialis* in tissues was also studied. Water analysis showed that TDCPP was stable in water during the course of the experiment and mussels bioaccumulated TDCPP rapidly, the bioconcentration factor being 150 L/Kg. Differentially expressed proteins upon exposure to TDCPP at both 7 and 28 days of exposure will be presented and discussed with the aim of gaining insight on the toxicological action of TDCPP and the cellular detoxification processes activated in mussels. Also, differential expression of AChE and GST proteins will be compared with the results of biochemical analysis of their activities.

TH090
Quantification of the amounts and PAH levels in different PM fractions deposited on roadside vegetation
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Particulate matter (PM) is a ubiquitous air pollutant formed by natural processes such as soil erosion or volcanic eruptions, but also anthropogenically by industrial plants and combustion processes. Major sources in densely populated conurbations include traffic exhaust (gases and soot particles) as well as tyre and brake abrasion. As the fine particles can penetrate deep into the lungs, they can cause health problems ranging from inflammation of the mucous membranes to cardiovascular diseases. Particles below 10 μm can penetrate into the bronchi, particles below 2.5 μm can penetrate into the bronchioles and alveoli and particles below 0.1 μm can even penetrate into the lung tissue and circulatory system. These health problems might be further exacerbated since the particles are loaded with various pollutants such as heavy metals or polycyclic aromatic hydrocarbons (PAH) depending on their source. Therefore, it is important to reduce these PM levels as much as possible. Here, the large surface area of urban vegetation can play a role due to its filtering effect. In this study, we investigated the amount of PM and associated PAHs retained by roadside vegetation (*Hedera helix*, ivy) next to a main thoroughfare in Aachen, Germany. Samples were collected from 5 sampling sites situated at different distances from the road. To investigate seasonal variation, samples were also taken at different time points. PM deposited on the leaf surface was collected by first washing with water, and in a second step with chloroform (to release PM bound in the cuticle wax layer). The PM was fractionated using three filter stages (10 μm, 2.5 μm and 0.2 μm) and gravimetrically quantified. Finally, the different fractions were extracted with Soxhlet and analysed for PAHs using GC-MS. SEM images of individual filters and leaves were taken to check the PM fractionation procedure as well as the particle composition.

TH091
RNA-Seq analysis of estuarine amphipods exposed to highway road dust
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Road dust is a very complicated mixture of chemical contaminants, originating from many types of sources such as motor vehicle exhaust, road pavement, tire wear particles, pesticides, and atmospheric deposition. Chemicals accumulated in the dust are potentially transferred into receiving waters by rainfall and sometimes cause adverse effects on water environments. Despite the extensive previous research efforts on road runoff toxicity, little is known about the mode of action of road dust toxicity in aquatic organisms. With an aim to reveal the biological effects of road dust on the estuarine amphipod *Grandidierella japonica*, we performed de novo transcriptome analysis of the amphipod exposed to road dust collected from highways around Tokyo. The transcriptome analysis by Illumina HiSeq 2500 identified a total of 97 transcripts differentially expressed by road dust exposure. These transcripts included the transcripts related to oxidative damage (e.g., peroxiredoxin), gamma-aminobutyric acid (GABA) signalling pathway, cuticle biosynthesis and metabolism, and transferase activity of

phosphorus containing chemicals (e.g., pyruvate kinase). Among these, the expression of $GABA_B$ receptor subunit was down-regulated in the road dust treatment but remained at the control level in the treatment of road dust with a carbonaceous resin XAD-4 which can reduce the acute toxicity of road dust to *G. japonica*. This indicates that the $GABA_B$ receptor expression was the biological response associated with acute lethality by road dust exposure. The expression changes of $GABA_B$ receptor and the related transcripts (e.g., glutamic acid decarboxylase, sodium- and chloride-dependent GABA transporter, GABA transaminase) were confirmed by additional quantitative PCR experiments. Although the linkage between the molecular initiating events by road dust exposure and acute lethality was still unclear, our findings in this study provide lines of evidence to understand the mode of action of road dust toxicity and to identify the causative toxicants in urban road dust.

TH092
Transfer of chemical compounds through ingestion of micronized tire wear particles in Mysida spp.
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Tire wear particles (TWP) constitute a significant contribution to particulate contamination of the aquatic environment. TWP are produced from driving, and may eventually be released to the surface waters, possibly together with leached granulated rubber particles used in artificial turf. Although rubber particles have been detected in wastewater treatment plants and in the aquatic environment, potential environmental impacts of this contamination and its effect on water-living organisms are largely unknown. In this present study we exposed field collected *Mysida spp.*, to varying dietary concentrations of TWP (0, 10 or 30% TWP) for 28 days. Initially, ingestion and depuration times of laboratory made micronized TWP (d< 250μm) were established. Hourly photos were obtained to track TWP in the stomach and gut, which was filled within 4 hours and emptied after 72 hours. The 28-day experiment was conducted with a daily 4-hour feeding window of 0, 10 or 30% dry weight TWP on top of regular diet (spirulina powder), after which all remaining particles were collected from the aquarium, to minimize leaching. Organisms were sampled for analysis on day 0, 14 and 28 to investigate possible chemical transfer of EPAs 16 priority PAHs, 10 tire-associated organic compounds of interest and a suite of metals, from gut lumen to tissue upon TWP ingestion. Results of the study will be discussed in this context and feed into the current debate on micro particle pollution.

Trans-Disciplinary Research on Coastal Ecosystems of the Northern Europe: Achievements and Problems (P)

TH093
Reclaiming an old fuel oil storage cave for producing sustainable energy utilizing the microbiological remediation process
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In order to ensure fuel safety, oil storage in caves has been a common practice in the Nordic countries for several decades. Due to the changes in fuel distribution and transportation, many of these caves have been emptied. The possibility to reuse these caves is challenging because residual oil is difficult to remove from the cave walls. However, if the oil can be eliminated, the caves can be reclaimed to produce sustainable, renewal energy as heat storages or by storing the energy of the sun heated seawater to be used in the cold winter months. This case study demonstrates the reclamation of an oil storage cave in Helsinki, Finland by utilizing the oil degrading bacteria naturally present in the sea water. The oil degrading bacterial communities of the Baltic Sea are known to be efficient oil degraders in optimal conditions, and could be utilized as the sole bioremediation agent. Sea water was collected in the late summer from Haakoninlahti bay in Helsinki, as well as water from a nearby decommissioned diesel oil storage cave in Kruunuvuori. The Kruunuvuori cave has a volume of 300 000 m^3, and is currently approximately one third full of oil contaminated groundwater. Oil degradation was tested in aquarium scale experiments corresponding to three scenarios: 1) oil degradation in the cave, 2) oil degradation in the cave if it is filled with seawater, and 3) oil degradation in the cave if it is filled with seawater and aerated. The C_{10}-C_{40} oil concentrations in the aquariums were measured at 0h, 1 week, 2 weeks, and 6 weeks. DNA samples were taken from each sampling to measureoil degradation genes PAH-RHDα GN, PAH-RHDαGP and AlkB by quantitative PCR. Bacterial V3-V4 and archaeal V4 regions of 16srDNA gene and fungal ITS1 regions from selected time points were sequenced by Ion Torrent for microbial community structure analysis. Our results show, that in six weeks 72% of the oil was degraded in the cave water in laboratory conditions. Mixing the cave water with 2/3 volume of sea water increased degradation to 96%, and mixing seawater and aeration to 96%. The copy numbers of PAH degradation genes were orders of magnitude lower than alkane degradation genes, indicating that alkanes are degraded rapidly. Our results show, that the residual oil is degraded naturally in the storage cave, but the addition of seawater enhances and accelerates the process.

TH094
Depth distribution of phototrophic bacteria, redox potential and hydrogen sulfide in highly stratified meromictic lakes of the White Sea region
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Ecological monitoring of sub-Arctic water bodies is essential for studying their evolution under the action of global climate change and other natural processes; it becomes extremely important when solving such practical problems as construction of tidal power plants, seawalls, bridges, and other hydraulic structures leading to the separation of water areas from the sea. As a result of isolation, the ecosystem of a saline water body undergoes irreversible changes: the surface water layer gradually becomes fresh and the near-bottom water stagnates with the formation of oxygen deficiency and hydrogen sulfide accumulaction. This leads not only to changes in the physicochemical characteristics of the water mass but also implies sharp changes in the microbial community which distribution can be monitored using optical spectroscopy. Along the coastline of the Kandalaksha Bay of the White Sea several lakes are known to be at different stage of separating from the Sea. We summarize the results of complex inspection of 5 meromictic lakes and lagoons performed in different seasons in 2014-2018. We sampled water from various depths and measured water temperature, salinity, pH, Eh, dissolved oxygen content, and illumination in the field. In laboratory conditions spectroscopic investigations of natural water samples and measurements of dissolved oxygen and sulfide anions (S2–) were carried out. The total Chl *d* and *e* concentrations were calculated from absorption spectra of pigment extractions in acetone:methanol mixture or directly from absorption spectra of intact bacterial cells in natural water using the recently developed technique. Spectroscopic methods allowed us not only to determine the presence of green sulfur bacteria (*Chlorobiaceae*), anoxygenic phototrophic bacteria, at different horizons but also to draw conclusions about relative concentrations of two forms of green sulfur bacteria (green-colored and brown colored) in each lake. Comparing the photosynthetic pigment concentrations with physicochemical parameters of water in the lakes made it possible to reveal favorable conditions for green sulfur bacteria: a layer with a sharp gradient of hydrogen sulfide concentration, salinity, and change of the redox potential from positive to negative. This study was supported by the Russian Foundation for Basic Research, project No 16-05-00548, and by the grant from the “Bazis” Foundation for Development of Theoretical Physics and Mathematics.

TH095
How was the Sour-Sweet Lake (Kandalkshsky Bay of the White Sea) born?
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During course-work practicums at the White Sea based on Nikolai Pertsov White Sea Biological Station, the second year students of Geomorphology and Paleogeography, Cartography and Geoinformatics departments of faculty of geography, Moscow State University, developed a method of integrated geomorphological mapping of the seabed, coastal zone and seaside land. The purpose of the work is to identify the mechanisms of transformation of the seabed in the coastal zone. On the northern coast of the Kindo Peninsula, a tacheometric and geomorphological survey and aerial photography of the coastal strip from an unmanned aerial vehicle (UAV) were carried out. A bathymetric survey was performed in the Rugozerskaya Bay, the Velikaya Salma Strait and the Kislo-Sladkoe Lake. The granulometric analysis of bottom sediments , tidal zone, beaches and coastal terraces was carried out. A combined digital elevation model (DTM) of the coast and the bottom, the geomorphological maps and orthophotoplans of key sections were compiled. The seabed relief of the glaciated shelves consists of structural-tectonic and glacial forms, partially modified by the processes of underwater morpholithogenesis. As a result of theglacioisostatic uplift of the coast the highest forms gradually fall within the wave impact zone. In the coastal zone, they undergo significant processing under the action of waves, tides, fast ice and biogenic processes manifesting in dramatic changes in the morphological characteristics and composition of sediments. Sea bays and straits turn to meromictic lakes and lagoons, and later to freshwater lakes and swamps. Mosaic-like morpholithodynamic situation control the time for specific sections of the coast to emerge from under sea level. At the semi-isolated lake Kislo-Sladkoe (Rugozerskaya bay, Kanadalaksha Bay, White Sea) northern strait formerly connected the lake with the sea was closed by sand spit created by the waves. The study allows to determine that the creation of the spit made the process of lake separation almost 150-250 years faster.

TH096
Spectroscopic characterization of CDOM in contrasting lakes Svetloe and Tyomnoe (Arkhangelsk region), extremely pure and extremely reach in humic substances
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The Arkhangelsk region is distinguished by a large number of lakes; according to estimates there are about 74000 water bodies. The majority of the lakes in the region were formed after disappearance of the last glacier, and some lakes have tectonic origin. Small lakes, abundant in the region, are especially significant from the viewpoint of a carbon biogeochemistry. The chromophoric dissolved organic matter (CDOM) is a complex mixture of organic biochemicals that undergo various pathways of diagenetic alteration. The CDOM absorbs UV and visible light and is a major determinant of optical properties for both fresh and marine waters, directly affecting the spectral quality of the underwater light field. The CDOM optical properties are currently used for monitoring of aquatic ecosystems. Fluorescence spectra of different CDOM types may vary in wavelength of emission maximum, fluorescence quantum yield values and their dependence upon excitation wavelength. We describe depth profiles of optical characteristics (absorbance, wavelength-derivated absorbance spectra, fluorescence emission and excitation spectra) for two contrasting freshwater lakes located in Arkhangelsk region: extremely pure (Svetloe Lake) and extremely reach (Tyomnoe Lake) in humic substances. Both studied lakes are located in boreal taiga within 100 km from Arkhangelsk. The Lake Svetloe is the first in a system of 5 lakes giving rise to the Svetlaya River, which belongs to the White Sea catchment area. Its maximum depth is 39 m; the area of the mirror is 0.125 km^2. The Lake Tyomnoe has maximum depth 37 m, the area of the mirror is 0.094 km^2. Both lakes are meromictic and belong to a specific type of water bodies intriguing researchers in many countries of the world. The chemocline is located at 20 m in Svetloe and at 25 m in Tyomnoe dividing water column in two zones with differing optical characteristics. The presence of chemical stratification, redox processes within the chemocline zone, contrasting optical properties lets us to consider those lakes as unique research objects for understanding both modern and ancient aquatic ecosystems. Those lakes, remote from major industrial centers, are especially interesting for the assessment of climate change effects as they are minimally influenced by acid rains and local anthropogenic impacts.

Linking Models, Experiments and Measurements to Reliably Investigate the Environmental Fate and Effects of Hydrophobic Organic Contaminants and Mixtures (P)

TH097
Determining the influence of street vegetation on PAH loads in the surface run-off from urban streets.
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Organic pollution in the environment is mainly caused by development and urbanisation. Especially in the city, many sources of pollution occur including power stations, factories or traffic. Polycyclic aromatic hydrocarbons (PAH) are a group of relevant and potentially carcinogenic organic pollutants with traffic being one important source. Here, the main PAH sources are exhaust gases, road and tyre wear, and leakage of motor oil. Gaseous as well as particle-bound PAHs get into the air, leading to exposure of the inhabitants. Further to the pollution of air by PAHs, they deposit to urban surfaces and are then washed off by rain into the sewer system. This leads to a potential contamination of surface waters and their sediments. Several studies have shown that vegetation can reduce pollutant loads via a filtering effect. Therefore, urban vegetation can potentially have a positive impact on health and quality of life. The aim of this work was to determine a possible impact of urban greening on PAHs in street run-off. In September 2018, run-off was collected at five locations with different amounts of vegetation along a main thoroughfare in Aachen, Germany using specially constructed sediment traps placed in the road drains. PAH concentrations in the water-phase were determined by both grab and passive sampling, and the particle-associated PAHs in the collected sediment. Total water flows were determined by coating the outer trap walls with plaster and measuring the weight loss. Additionally, the sediment was analysed with a CHN analyser to determine the natural organic matter and soot amounts. PAH concentrations and profiles were compared for the different street sections and related to the different amounts of vegetation.

TH098
Liposome mediated delivery of challenging organic chemicals to Daphnia magna
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Liposomes are phospholipid vesicles widely used in drug delivery, shown to be beneficial for stabilizing the delivery of therapeutic compounds.[1] It has been observed that giant liposomes (6-7 µm) can be efficiently taken up as food particles by filter feeders when they share the same size range as their food.[2,3] Giant liposomes can be used as a delivery system of a wide range of organic chemicals: hydrophobic chemicals can be trapped within the lipid bilayer, whereas hydrophilic and ionic compounds can be encapsulated in the aqueous pocket.[1,4] This offers the advantage to study challenging chemicals for aquatic toxicity testing, when the use of a solvent carrier does not allow a controlled exposure to the toxicant. Moreover, their natural buoyancy and composition (non-toxic, digestible components), adds to the advantages of using such approach in this context. In this study, we prepared large phospholipid vesicles in synthetic freshwater (M7 medium). Liposomes were prepared with a mixture of phosphatidylcholine and cholesterol, and a final average size of 6.1 ± 0.44 µm. Staining of the liposomes with the fluorescent dye Nile Red allowed us to confirm the ingestion of liposomes by *D. magna*,viewed under the microscope. Uptake rate of liposome particles was also measured and compared with the uptake rate of algae cells. Thereafter, liposomes were prepared with 1% (w/w) ^{14}C-labelled chemicals, to evaluate the encapsulation yield. The chemicals tested were tetrabromobisphenol A (TBBPA, log K_{ow} 5.9, pK_a 7.5), medium-chain chlorinated paraffin CP-52 (log K_{ow} 8-12) and the amphiphile perfluorooctanoic acid (PFOA, pK_a 2.8). When the chemical-specific encapsulation yield was known, animal experiments were conducted to evaluate the survival of *D. magna* after aqueous or liposome-mediated chemical exposure. Survival of the neonates (2 mL water/ animal) was followed for 48 hours, as according to the OECD guideline 202. Several concentrations were used by manipulating the ratio of dosed and chemical free liposomes. The major aims of this work was to i) establish differences in internal dose of the different chemicals via aqueous or liposome-mediated exposure, ii) compare the dose-response curves, and iii) ultimately, validate the use of liposomes for as a controlled delivery method for challenging chemicals.
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TH099
Predicting mobility of hydrophobic organic chemicals in soil: exploring limits and reliability of multimedia fate models
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A soil column leaching experiment was performed to investigate the effect of many influential variables on vertical transport of polychlorinated biphenyls (PCBs) in an aged contaminated soil. The factors investigated were: 1) contact time between soil and leaching solution (2, 5, 7, 48 days), 2) dissolved organic carbon (DOC) content in leaching solution (tap water *vs.* Aldrich humic acid), 3) temperature (25 °C *vs.* 15 °C) and 4) soil saturation conditions (saturated *vs.* field capacity conditions). Additionally, the transport mediated by mobile organic carbon (OC) coated fine particles/colloids was accounted for. Measured bulk water concentrations were compared to data predicted using a multimedia environmental fate model (SoilPlusVeg) to explore model performance, limits and predictive capability. The factors instigated had a relevant influence on DOC and particle production and movement, with significant variations in PCB leachate concentrations (up to 1 order of magnitude), generally not captured by model simulations. Environmental fate models substantially mismatched predicted and observed concentrations, especially for the high chlorinated congeners because some key features are missing: 1) the transport of chemicals associated to small particles/colloids; 2) the modulation of the production of DOC/particles varying with temperature, soil/water contact time and environmental conditions; 3) the chemical sorption/desorption kinetics.

TH100
Experimental measurements and modeling assessment of snow scavenging of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5)
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Snow scavenging of two volatile methylsiloxanes, octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), was examined by a combination of laboratory measurements and modeling assessment. In the experimental study, snow sorption coefficients of ^{14}C-labeled D4 and D5 on snowflakes (K_{iA}) were measured against a benchmark compound, cyclopentanone, at different temperatures varying from -2 to -20 °C using a custom-made snow chamber. In addition, the transfer of D4 and D5 in snow to surface media was measured in simulated snow melting experiments using ^{14}C-D4 and -D5 spiked snowpacks (30 cm thick) placed on top of a frozen soil layer (10 cm thick) under controlled air flow rates (representing wind speeds). In the modeling assessments, the snow scavenging effects in Polar Regions with yearly snow-melting processes were evaluated with the measured K_{iA} values and other physicochemical properties such as air/water (K_{AW}) and octanol/air (K_{OA}) partition coefficients and their temperature dependences, and the transfer efficiency of D4 and D5 in snow melting processes. It was found that the gas scavenging of D4 and D5 was small because of their small K_{iA} values, while the particle scavenging is negligible due

to D4 and D5's small K_{OA} values (thus small K_P values) together with low aerosol concentrations in the Polar atmosphere. Most importantly, almost 99% of all D4 and D5 in a snowpack was lost during the snow melting process and D4 and D5 became non-detectable in snowmelt water and surrounding soil. In short, snow scavenging is not an effective deposition mechanism for these volatile hydrophobic compounds.

TH101

Mass Balance of Atmospheric PAH at Alpine Lysimeter

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Polycyclic aromatic hydrocarbons (PAH) are well known ubiquitous organic contaminants, which are emitted to a huge extent into the atmosphere (emissions of EPA-PAH benzo(a)pyrene in 2016 in Germany ca. 30 t/a). Little is known about atmospheric deposition and mass balances in alpine regions. Meyer et al. (2006) showed a PAH flush release in snow lab experiments simulating cold environments. The ecological impact, particularly at e.g. eco-sensitive alpine regions is unknown. This study is aiming the quantification and mass balance of atmospheric PAH in alpine soil lysimeter from precipitation, soil and seepage samples. The 2 lysimeter are located at Mount Stoderzinken (1800 m amsl) and Gumpenstein village (696 m amsl) in the Northern Limestone Alps in Austria. All investigated PAH could be quantified in the soil samples, but at different concentrations. At Gumpenstein in the same order of magnitude at all sampling sites, but not at Stoderzinken (remarkable differences from site to site). Usually heavy PAH (molecular weight MW > 202) were enriched. During summer, PAH concentrationsin water samples (precipitation, seepage) with MW < 202 were in the same order of magnitude. PAH with MW > 202 were detected in precipitation samples, but not in snow samples. PAH mass balances were calculated for May/June and autumn 2016 at both lysimeter showing higher input rates at Stoderzinken. At Stoderzinken for 6 detected PAH (naphthalene Nap, fluorene Fln, benzo(b)fluoranthene BbF, benzo(e)pyrene BeP, phenanthrene Phe, pyrene Pyr) the mass balance indicated an accumulation in soil for May/June for Nap, Fln, BbF und BeP. A release was found for Phe, Pyr und Fln. We assumea particle and or colloid transport due to the low water solubility and high sorption coefficient of those compounds. Lysimeter Gumpenstein showed for May/June an input into soil for Fln and Pyr and output of Nap, Fln and Pyr. In September 2016 Nap, Fln, Phe and Pyr were deposited at Stoderzinken as well (no BeP and BbF), in addition benzo(a)pyrene was accumulated. Nap showed higher abundance compared to June/May and was detected in the output as well. At Gumpenstein it was possible to balance from Oktober to December 2016, with similar results like at Stoderzinken in September: High Nap in- and output, input only for PAH with MW > 202. We assume the heating season for the different results in summer and autumn/winter.

TH102

Bisphenol A study in rainbow trout (Oncorhynchus mykiss) at elevated environmental concentrations

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A study on rainbow trout (*Oncorhynchus mykiss*) fingerling weighted from 10–20 g was conducted in the laboratory. For 20 days fish were exposed to nominal water concentration of bisphenol A (BPA) of 1 µg/l, 20 µg/l and 0 µg/l for the first, second and third group, respectively. To ensure adequate water quality while maintaining tested concentrations of BPA in a closed water system fish were moved between two aquariums in one and two-day intervals. In the study every second day, seven fish per group were sacrificed, dissected and muscle and skin tissue samples were collected and pooled. In addition, water samples were taken before and after fish were settled in the aquaria. Aquaria water and muscle tissue samples were analysed for the presence of BPA by either isocratic or gradient reversed-phase HPLC using water and acetonitrile as a mobile phase, Hypersil Gold C18 (3 µm) analytical column, and fluorescence detection. Aquaria water samples were measured either directly, after proper dilution, or with a pre-concentration using solid phase extraction (SPE) phase Chromabond HR-X. Homogenised muscle tissue samples were extracted with acetonitrile and purified with the two SPE phases, at first with Chromabond HR-X and secondly with (MIP) AFFINIMIP® SPE Bisphenol A. Limit of detection values of BPA determination were 0.1–0.5 µg/l for aquaria water and 1 µg/kg for fish muscle and skin in natural proportions. The results showed that the deviation values of the measured exposure concentrations from the nominal concentrations were low, on average by –14.8 and 1.7% for the group exposed to 1 and 20 µg BPA/l, respectively. Breakdown of BPA in water with fish was in correlation with the number of fish in aquaria and duration of inhabitation period. After one-day settlement with fish it was 41 and 54% on average, and after two-day settlement, concentrations of BPA dissipated on average for additional 28 and 9% for the group exposed to 1 and 20 µg BPA/l, respectively. On the wet weight basis, concentrations of BPA in the fish muscle and skin tissue, ranged from 3.3 µg/kg (days 14 and 16) to 7.6 µg/kg (day 2) for the group exposed to 1 µg BPA/l, and from 18.3 µg/kg (day 4) to 75.5 µg/kg (day 20) for the group exposed to 20 µg BPA/l. The presented study aimed to contribute to understanding the BPA exposure concentrations and its bioaccumulation in the environment.

TH103

Passive sampling of traffic-derived dissolved pollutants in street run-off from urban areas with different amounts of vegetation

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The constantly rising demand for mobility is accompanied by increasing emissions of traffic-derived pollutants. This causes problems, especially in urban areas where traffic is high, air exchange low and the surfaces predominantly sealed. Considerable amounts of the emitted contaminants deposit to urban surfaces, where they can be re-dispersed or get flushed into street drains during rain events. Particularly during heavy rain, untreated run-off can therefore directly enter surface waters. An important role in managing rising pollutant emissions can be played by urban green, with the huge surface of the foliage and the soil underneath offering the potential for filtering and binding the pollutants, leading to a lower bioavailability.\n \n To further investigate the influence of urban vegetation, a field study was conducted along a busy ring road in Aachen city (Germany) with greened and non-greened sections. Over one month, surface run-off was sampled in five street drains equipped with passive samplers for the determination of time weighted average (TWA) concentrations of the traffic-derived dissolved pollutants. The samplers were used in kinetic mode with the hydrodynamic conditions varying strongly between dry and rainy periods. Therefore, a robust sampler calibration was necessary and two methods were compared (i) a new approach using the concentration ratios of samplers with different thicknesses and (ii) an established approach based on the release rates of performance reference compounds. Polycyclic aromatic hydrocarbons were chosen as marker substances, since they show a wide range of physical-chemical properties, thus allowing for extrapolation to other traffic pollutants.

TH104

Impact of soil conditions and simultaneous application of 6 pharmaceuticals on their sorption and degradation in 7 soils

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The leakage of pharmaceuticals present in soils towards groundwater and their uptake by plants are largely controlled by sorption and degradation of those compounds in soils. Standard laboratory batch sorption and degradation experiments were performed using soil samples taken from top horizons of seven different soil types (Stagnic Chernozem Siltic, Haplic Chernozem, Greyic Phaeozem, Haplic Luvisol, Arenosol Epieutric, Haplic Cambisol, Dystric Cambisol) and 6 pharmaceuticals (carbamazepine, citalopram, clindamycin, fexofenadine, irbesartan and sulfamethoxazole), which were applied either in single-solute solutions or their mixture. The sorption affinity of different compounds depended on their form and particular soil properties. The sorption of positively charged compounds (citalopram and clindamycin) increased with the increasing base cation saturation of soils. The sorption neutral and negatively charged (sulfamethoxazole and irbesartan) and zwitter-ionic (fexofenadine) molecules decreased with the increasing base cation saturation of soils. Sorption of carbamazepine depended on the organic carbon content. In soils of the high pH and high base cation saturation, the largest sorption was obtained for citalopram, followed by fexofenadine, clindamycin, carbamazepine, irbesartan and sulfamethoxazole. In soils of the low pH and low base cation saturation, the irbesartan sorption affinity was between fexofenadine and clindamycin sorption affinities. The simultaneous applications of all pharmaceuticals slightly decreased sorption of carbamazepine, and clindamycin, and increased sorption of fexofenadine, irbesartan and sulfamethoxazole. This documented completion of some compounds for the same sorption sites but also compound synergy. The compound degradations depended overall soil conditions that affected microbial activities in soils. The highest half-lives were observed for citalopram followed by carbamazepine, fexofenadine, irbesartan, clindamycin and sulfamethoxazole. The simultaneous application of all compounds increased the half-lives of carbamazepine citalopram, fexofenadine and irbesartan, which can be likely explained by the negative impact of antibiotics (sulfamethoxazole and clindamycin) on soil microbial activity.

TH105

Why and what scientists should know about intercomparison studies?

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Analytical test results are used to test, monitor, assess, and model the state of environment. The reliability of any result used depends on the quality of the whole chain of its production. The end user of any numerical or qualitative result must rely on the proficiency of that chain. For environmental data the chain covers the field observations and sampling as well as data production in environmental laboratories. One way for the data producer to demonstrate its competence is to take part in intercomparison studies. Intercomparison studies are comparisons performed in accordance with predetermined conditions by two or more participants. The competence of individual participants is evaluated by the objective scheme, including measurements of the same or comparable tasks. Intercomparison studies provide an effective external way to assess participant's proficiency to produce certain information and whether the participant's procedures are fit for purpose. Participants can use the results to confirm the quality of their measurements and to compare their results to those of other participants, also they may promote their quality to their customers. Proftest SYKE provides a wide range of intercomparison studies for analysis of environmental samples and recycled materials. The open access reports of Proftest SYKE intercomparison studies are published at the Digital Repository of the University of Helsinki. The results are presented anonymously, but the participants are listed in each report. The publicly available reports are found to be important not only for the participants but also for a much wider audience. Different authorities responsible of e.g. monitoring data on the area of environmental health, accreditation bodies and various researchers exploit the open access of the reports. End user of the analytical test results should understand the basics of the quality assurance procedures. Scientists and other end users of the test results could request and obtain the quality information related to the provided data from the data producer, and utilize the information when employing the data. Participation in the intercomparison studies and documented good performance in them is evidence of satisfactory quality of the results. Reference: www.syke.fi/proftest/en

TH106

Can long-range atmospheric transport events of "new" POPs to a remote site in Norway be predicted using FLEXPART?

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Air monitoring of organic contaminants with suspected POP properties at background sites helps to assess their long-range atmospheric transport (LRAT) potential. The most accurate approach is active sampling but this is often limited to intermittent collection, e.g. one day per week. Regarding LRAT, such sampling strategies may be incomplete if the sampling schedule misses episodes of heavy influence by LRAT. Despite their low occurrence, these episodes can have a significant effect on the overall pollutant amounts transported to remote sites. The Lagrangian particle dispersion model FLEXPART was applied earlier to successfully forecast LRAT-dominated episodes for polychlorinated biphenyls (PCBs). Using emission data for PCB-28 as model input, it predicted the relative temporal variation of concentrations equally well for other PCB congeners too. Unfortunately, for many other compounds emission estimates are not available, e.g. for some industrial organic contaminants of emerging interest. Our aim is to assess whether possible spikes in concentrations of such chemicals can be predicted, detected and potentially explained by LRAT, assuming similarities in source regions as for the PCBs. Targeted air sampling was carried out at the Birkenes observatory (southern Norway) over 24 h each, using a high volume active sampler at a sampling rate of ca. 50 m^3/h. Samples were collected during periods when the model-based forecasted air mass transport path, and the resulting predicted concentration of PCB-28, indicated potentially interesting events. The samples will be analysed for compounds suspected to behave similar to PCBs and/or which have only very recently been added to Annex A of the Stockholm Convention, such as chlorinated paraffins, dechlorane plus and selected brominated flame retardants. To help with the interpretation of the data we will also analyse compounds with already identified LRAT potential, such as PCBs and PBDEs. The results will help to evaluate whether the selected contaminants of emerging interest undergo LRAT and where their source areas are. If FLEXPART can predict concentration variations sufficiently well, then a model-based sampling strategy may complement intermittent monitoring strategies and provide valuable information about atmospheric source-receptor relationships.

TH107

Environmental fate and behaviour of thifensulfuron methyl metabolites

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Nowadays, herbicides are widely used for the purposes of weed control in households and arable field spraying in agricultural production. Therefore, there is a risk that active substance residues and metabolites may leak into the soil. The presence of pesticide residues in the soil can cause damage to sensitive follow-up plants. Residues of pesticides can also get into groundwater. Hence, it is extremely important to determine the dynamics of pesticide degradation in the soil. For this study thifensulfuron methyl metabolites were selected. The reason for it was their common occurrence in various herbicides commercially available in Poland. This herbicide is used as a post-emergence herbicide for the control of grass and broad-leaved weeds. Thifensulfuron methyl is commonly used in wheat, barley, triticale, rye and maize crops. This study was aimed at determining the rate of degradation of thifensulfuron methyl metabolites in three types of soils commonly found in Europe. The DT_{50}, DT_{75} and DT_{90} values represent the time within which the concentration of thifensulfuron methyl metabolites is reduced by 50%, 75%, and 90 %, respectively. Other aims of the study were to assess the adsorption and desorption behaviour of thifensulfuron methyl metabolites on five different soil types and to determine the K_d, K_{oc}, K_{Fads}, and K_{des}. Based on the obtained results, it was shown that the dynamics of degradation of the investigated metabolites of thifensulfuron methyl depended on the soil (i.e. the amount of organic carbon, microorganisms biomass, or the pH level). It was also shown that these metabolites had different affinities for soils.

TH108

First results from the AQUA-GAPS program for global monitoring of POP

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Under the Stockholm Convention on persistent organic pollutants (POP), the Global Monitoring Plan (GMP) collects data on POP levels in human milk and air, but not in the aqueous environment. Inspired by GAPS (Global Air Passive Sampling), an international group of scientists meeting at Jinan University (2016, Guangzhou, China), started a passive sampling program in the aqueous environment, each using their network to expose passive samplers all over the globe, giving birth to "the AQUA-GAPS initiative". Indeed, monitoring POP in water on global scale is a (logistical) challenge, but has become feasible with the further maturation of aqueous passive sampling procedures. The resulting freely dissolved concentration have little confounding factors allowing comparison on a global scale. Instead of merely reporting freely dissolved concentrations, passive sampler data can also be converted to lipid basis, which represents a relevant indicator for the exposure conditions for aquatic organisms. Besides providing data for assessing spatial and temporal trends of POP, AQUA-GAPS will also act as a platform for expertise exchange and assisting participants that want to extend the local spatial coverage. Hardware for sampler deployments was manufactured and passive samplers, both silicone and LDPE, were prepared and send to participants in the network. Presently AQUA-GAPS covers approximately 30 sampling sites distributed over North and South America, Europe, Africa, Australia and Asia. Samplers were analyzed at RECETOX, Masaryk University, Czech Republic and from the uptake aqueous and lipid based concentrations of various compounds were derived. These are polycyclic aromatic hydrocarbons, polychlorinated biphenyls, various chlorinated pesticides, organo-phosphate and brominated flame retardants. Other pollutants of global concern will be included over the course of time when samplers and analytical methods become available. In this poster the first results will be presented showing spatial occurrence of the listed compound groups.

TH109

Recommendations for the monitoring of priority substances in freshwater fish based on practical experiences

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The Water Framework Directive (WFD) and in particular Directive 2013/39/EU set requirements for monitoring of environmental quality standards (EQS). For compounds, which tend to bioaccumulate in biota and show a risk of secondary poisoning or for human health by fish consumption, EQS were derived for biota (fish, mollusc and shellfish). To support the implementation of biota monitoring in Germany a research project was initiated with the aim to integrate all legal requirements into an appropriate monitoring concept. A comprehensive sampling campaign including six sampling locations (rivers, lake and a lagoon at the Baltic Sea) and three fish species with twenty individuals per species was conducted. Beside biometric data, concentrations of total mercury and organic substances such as perfluorooctanesulfonate (PFOS), brominated diphenyl ethers (BDE) or hexachlorobenzene were determined in pooled fillet and carcass samples. This data set allows the evaluation according to fish species, age, size, weight, sex and trophic level. Furthermore, the concentrations in fish fillet and whole fish were compared as the use of fillet samples better reflects protection of human health via fish consumption, which is the protection goal for five of the nine biota EQS for fish. Based on the results of this study, recommendations for future monitoring are given. Special focus is laid on a good compromise between scientific justification and aspects of practical implementation.

TH110
Studying desorption of additives from polymers using quartz crystal microbalance
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Various additives, including plasticizers, flame retardants, and UV filters, are incorporated into virgin polymers to improve their properties and enhance their processabilities. These additives are not bound to polymers and can be released from the matrix to the surroundings during their production, use, recycling or disposal phase. These chemicals could pose a hazard and cause adverse effects on human health and the environment. It is of great importance to study desorption mechanisms to increase our understanding of chemicals fate in products and the environment. Quartz crystal microbalance (QCM) is a fast and highly sensitive tool for environmental research at solid/liquid interface with the advantage of high accuracy (*i.e.*, ng cm^{-2}) in real time, which is rarely used for studying desorption processes of additives. The aim of this research was to study desorption characteristics of additives from various polymers using QCM and to predict their emission rates. The specific aims were: (1) to develop a fast and highly sensitive system to measure desorption kinetics of additives in real time; (2) to study effects on desorption of factors, such as polymer properties, temperature, pH, and salinity; and (3) to establish desorption kinetic models for predicting emission rates of various additives. Commonly used polymers, including polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET), and poly(methyl methacrylate) (PMMA), were selected in our study. They differ in e.g. hydrophobicity, crystallinity, glass transition temperature, and hydrogen bonding properties, which affect desorption of additives. Virgin polymeric materials were blended with selected additives, which octanol-water partition coefficient (log K_{ow}) values ranged from 1.6 to 7.0. The composites including PVC, PS, PMMA, or PET were coated on QCM electrodes to measure desorption in real time. We found that desorption of additives can reach steady-state in less than 12 hours, which differed depending on studied polymers, log K_{ow} value of additives, and loading content. Additives with high log K_{ow} value or blended with hydrophobic polymers showed lower desorption rates. In a next step, contact angles and crystallinity of polymer films will be measured to increase our understanding on desorption behaviors of additives from a molecular interaction perspective. Furthermore, desorption data will be used to establish kinetic models to predict emission rates of ranges of additives.

TH111
Development of Multimedia Fate Model for Human Risk Assessment of EDCs in the South Korea
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Multimedia fate model, HURAME (multimedia fate model for HUman Risk Assessment in Multimedia Environment) has been developed considering topographic features and environmental and been meteorological data of watershed region of South Korea. Human risk assessment system using this multimedia fate model was established and integrated to assess spatial exposure and risk about human living in this area. For this purpose, first, GIS database system consisting of environmental and meteorological data and measured data of hazardous chemicals was developed. Second, Environmental concentrations of various EDCs (Endocrine Disrupting Chemicals) were predicted, applying different fate processes according to different chemical properties. Third, advection and dispersion by wind in air grids, runoff in watershed, flows of water in water segments are considered in the watershed-based multimedia fate model, which was linked to a risk assessment system that predicts environmental multimedia concentrations of EDCs and assesses human risk in this area. HURAME is valuable tools for predicting the fate of chemicals in evaluative and real environments with areas consisting of many watersheds. These models are an integral component of exposure assessment and risk assessment strategies, and are used in detailed assessments of contaminant fate. The aim of these models is to describe quantitatively contaminant fate and migration in a defined watershed region, with water segments and air grids inside of the region of interest treated more complicatedly. As a result, regional levels of environmental contamination are controlled by environmental parameters and processes and meaningful evaluation requires assessment of contaminant fate in neighboring regions. A linked set of regional models thus has the potential to describe quantitatively the impact of chemical emissions over a wider geographic scale with significant spatial differences in environmental characteristics and chemical use patterns.

TH112
Occurrences, sources, and transport of organophosphorus flame retardants (PFRs) and persistent bioaccumulative toxic (PBT) contaminants in polar seas
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Organic pollutants in the polar seas have given rise to ongoing public concern, which also provide a good opportunity to study the spatial transport and temporal accumulation of environmental contaminants and the impacts of anthropogenic activities. However, the vast dynamic marine water poses challenges to their comprehensive monitoring within appropriate spatial and temporal scales. In this study, an combined approach of time integrated and on-board passive sampling of organic pollutants using triolein-embedded cellulose acetate membranes (TECAMs) was performed in waters of Fildes Peninsula, Antarctica and an Arctic cruise. The TECAM extracts were used for target analysis of organophosphorus flame retardants (PFRs), and non-target screening of persistent, bioaccumulative, and toxic (PBT) contaminants using two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). In the non-target analysis, 16 PBT contaminants were screened out from 1500 suspected compounds and performed with further confirmation. Multiple local sources (wastewater, air traffic, research stations, and animal feces) for different PFRs in waters of Fildes Peninsula were proposed, whereas both West Spitsbergen Current (WSC) and local sources (settlements, oil exploitation, and petroleum fuel emissions) were found to contribute to the PBT pollution in Arctic sea. Furthermore, a comparison among water sampling cases revealed that passive sampling can be a tool for aquatic time-integrated investigations in polar seas. Finally, annual transport fluxes of the contaminants from the North Atlantic to Arctic Ocean by WSC were estimated and their hazard to Arctic should be concerned.

TH113
Partitioning based determinations of water solubility for highly hydrophobic liquid chemicals
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Water solubility is a fundamental parameter for the environmental risk assessment of organic chemicals. It is experimentally and analytically challenging to determine the aqueous solubility for highly hydrophobic liquid chemicals, because they are prone to the formation of micro-droplets and emulsions in the saturated water. Two partitioning based methods were therefore recently developed for saturating water while avoiding direct contact with the hydrophobic liquid test substances. The first method is based on first saturating a silicone rod with the test substance and then using the silicone rod for saturating the water. The second method is based on saturating water from the liquid phase chemical via the headspace. Equilibrium kinetics of the headspace dosing method was determined in order to explore its applicability domain. Headspace passive dosing of 5 chemicals with log K_{ow} of 4-6 and K_{aw} of 0.8-200 reached 95% of equilibrium within a few hours. Headspace passive dosing was very precise and well-behaving for several of the test compounds, but challenges remained for chemicals with a log $K_{ow} > 6$ that are most prone to the formation of micro-droplets, emulsions and also other potential artefacts. A third passive dosing format has thus been developed and will be tested aiming for increased robustness of solubility determinations for highly hydrophobic liquid chemicals of log$K_{ow} > 6$. Finally, the solubility of several model compounds will be determined in pure water and five other media such as algae toxicity test medium, lake water and seawater.

TH114
Investigating PCB mobility in soil water after a rhizoremediation experiment: role of different plant species and soil conditions
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The study of organic contaminant mobility through soil profile is important to understand their environmental fate. This can be achieved by both field and laboratory experiments. Among laboratory tests, column leaching experiments could allow to investigate organic contaminant movement due to a water flux that simulate an irrigation or a rain event under more realistic conditions. The aim of the present work was to investigate the mobility of PCB in soil performing a laboratory experiment with glass column filled with an aged highly contaminated soil (mg/kg level). The soil was collected in a National Relevance Site for remediation located in Northern Italy (SIN Brescia-Caffaro) and has undergone a rhizoremediation treatments (P) for 18 months with different plant species (*Festuca arundinacea* and *Cucurbita pepo ssp pep*o in consociation (P4) and *Medicago sativa* withmycorrhizal fungi (P7)). The same but unplanted soil was also considered as control (C) for comparison. Three replicates were considered for each type of soil. The columns were leached with tap water and PCB concentrations were measured in the leachate after 7 days of contact. The samples were also characterized for pH, conductivity, temperature and dissolved organic carbon (DOC) content. Total PCB concentrations (sum of 84 congeners) ranged

from 15 to 42 ng/L in the dissolved phase and from 5 to 135 ng/L in the particle phase, representing a small fraction of the total amount in soil. Leachate samples were enriched in tetra to hepta PCBs, while deca PCB showed a lower contribution to the fingerprint considering bulk concentration; mono, di, octa and nona PCB concentrations were below the detection limit due to their low concentrations in soil. Both treatments (P4 and P7) showed similar PCB dissolved concentration with respect to the control, while water leached from soil treated with *Medicago sativa* (P7) showed PCB particle phase concentrations a factor of about 2 higher than those of the control and the other treatment (P4). This indicated that the selected plant species, during the rhizoremediation treatment, acted differently on one of the most important fluxes influencing chemical movement in soil (POC, particle organic carbon).

TH115

Bioaccumulation of brominated flame retardants (BFRs) by two freshwater invertebrates exposed through different routes.

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Brominated flame retardants (BFRs) have been a major concern for several decades due to their intensive use in commercial, consumer and household products. The contamination of various environmental samples has been recognized worldwide. As a result, regulations were adopted, in order to restrict or ban their production and use. Nevertheless, some BFRs are still detected in various invertebrates and fish species, such as hexabromocyclododecane (HBCD) and penta and octa-BDEs, mainly represented by BDE-47 and BDE-99. Debromination mechanism leading from BDE-99 to BDE-47 have been demonstrated in some fish species. Additionally, previous studies suggested an isomerization of HBCD from sediment (mainly γ-HBCD) to fish (α-HBCD). To a better understanding of the fate of BFRs in aquatic organisms, the evaluation of the transfer of these compounds from sediment to invertebrates is required, since sediment serves as a sink for these pollutants. Toxicokinetic (TK) models have been developed to describe the accumulation of contaminants in aquatic food webs. TK model parameters could be easily determined by short-term exposures of organisms under controlled laboratory conditions. To date, few data are available on the bioaccumulation kinetics of BFRs in freshwater invertebrates. Chironomids and gammarids are ubiquitous in European freshwaters, easily sampled in the field and they play important ecological roles as a link between the base of food webs and higher-order consumers such as fish or birds. Besides, these two organisms have been reported to accumulate metals and various organic pollutants. The aims of this study were (i) to investigate the potential biotransformation of BDE-99 in to BDE-47 and the potential isomerization of γ-HBCD to α- or β-HBCD in invertebrates and (ii) to describe the related bioaccumulation kinetics. *Gammarus fossarum* were exposed in aquatic microcosms to BDE-99 spiked leaves and clean sediment, while *Chironomus riparius* and *G. fossarum* were exposed to HBCD spiked sediment. Thus, internal levels of chemicals were regularly measured to estimate uptake and elimination rates.

TH116

Influence of co-dosed lipids from biota extracts on the availability of chemicals in in vitro cell-based bioassays

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Bioanalytical tools are widely used for assessing the burden of chemical mixtures in the environment including biota. However, interpretation of bioanalytical data from biota samples can be challenging because of matrix effects caused by co-extracted lipids or proteins. In cell-based reporter gene assays, these matrix residues are expected to have a substantial effect on the read-out quality. We consider co-extracted lipids to act as an additional phase in the bioassay system because lipids do not dissolve and are not likely to be taken up by cells. This additional lipid phase is expected to reduce the bioavailability of dosed chemicals, thereby reducing the apparent sensitivity of the assay. We can describe partitioning of diverse chemicals between co-extracted lipids, medium components and cells by a simple equilibrium-partitioning mass-balance model that is parameterized with (a) the partition constants between lipid and water, medium and water as well as cells and water, (b) the constituents of medium and cells in terms of volume fractions of lipids, proteins and water and (c) the volume fraction of co-extracted lipids. We used triolein, a synthetic triglyceride often used as a standard lipid, to mimic the co-dosed lipid matrix. We measured the dependence of the effects on triolein in the well-characterized cell lines AhR-CALUX for activation of the aryl hydrocarbon receptor and AREc32 that responds to Nrf2-dependent oxidative stress. Triolein was spiked with two bioactive reference compounds for each assay with different log K_{OW} and dosed directly to the cell assays to cover triolein volumes between 0.5 and 4%. For a proof of principle the applied triolein volumes were much higher than realistic co-extracted lipids (in the extract typically

TH117

Novel methods for assessing the environmental risk caused by the reuse of sewage sludge

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Sewage sludge is an inevitable byproduct produced in waste water treatment process. Sludge is rich in nutrients, and bringing them back to natural circulation would diminish the amount of waste ending to soil dumping areas and promote the circular economy. However, during the sewage treatment process several harmful organic chemicals are retained in the sludge which raises concerns towards its use in agriculture and landscaping. Proving the safety of processed reused sludge would encourage its more extensive use. Sewage sludge is a complex matrix including a number of organic and inorganic compounds. Due to complexity of the matrix, the analyses of chemicals in municipal sewage sludge are not straightforward. Total concentrations of harmful chemicals include both dissolved and particle-bound fraction which may overestimate the actual toxic effects. The most harmful chemical fraction is the dissolved one which is able to transport in the environment and accumulate to organisms. This fraction can be studied with passive sampling. In this study passive sampling was combined with chemical and ecotoxicological examinations to indicate the readily bioavailable part of the chemical and assess the toxicity after digestion and composting. The polydimethyl siloxane (PDMS) passive samplers were deployed in untreated, digested and composted sludge. The toxicity of the extract was studied with chronic toxicity test with *Daphnia magna*, neutral red retention assay and umuC assay. The concentrations of selected chemicals such as polyaromatic hydrocarbons (PAHs), triclosan (TCS), and methyl triclosan (mTCS) were determined in sludge as well as in PDMS passive samplers. Digestion decreased both cytotoxicity and genotoxicity. This indicates that some of the compounds causing cytotoxicity are more efficiently removed in digested sludge compared to untreated sludge. The PDMS passive sampling method can be applied to estimate the bioavailable chemical fraction in waste water treatment plant (WWTP) sludge. Assessing the toxicity of sludge based on different bio assays can provide a more comprehensive picture of its true harmfulness to organisms. The treatment stages of municipal sewage sludge, especially composting, reduce toxicity and amount of studied chemicals. Nevertheless, based on the chemical analysis a rather large part remains in the sludge.

TH118

Fluorescent probes for measuring toxic effects in Daphnia magna

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The toxicity of chemicals to aquatic organisms such as *Daphnia magna* is often evaluated using dichotomous endpoints such as immobility and/or mortality. However, changes occurring at the biochemical level can indicate early responses to toxicant stress. This study investigated sublethal effects of common toxicants to *D. magna* using a novel fluorescence based assay for measuring in vivo enzyme activity. The study examined the hydrolysis of fluorescein diacetate (3,6-diacetoxyfluoran) as a measure of hydrolase activity in *D. magna* after exposure to toxicant stress. Live *D. magna* rapidly hydrolyze fluorescein diacetate (FDA) to fluorescein in aqueous solution resulting in distinct changes in absorbance (color) and fluorescence in the surrounding medium. The hydrolase activity in *D. magna* was proportional to animal biomass, incubation time and viability of the animals. Dose-dependent changes in hydrolase activity was observed after exposure to a range of potential toxicants including glyphosate, bisphenol A, fluoxetine, propranolol, β-estradiol, copper, chromium and UV A radiation. Changes in hydrolase enzyme activity in *D. magna* compared favorably to changes in mobility resulting in comparable or lower EC values. The FDA assay is simple and cost effective compared to other staining techniques. The fluorescein product provides a visual cue (green color) and green fluorescence that can be visualized and quantified using standard instrumentation (microscopes and plate readers). Hydrolase activity may be quantified as a combination of whole animal fluorescence and fluorescence in the surrounding water. The results suggest that the FDA assay can be used as an index of toxicant stress in *D. magna* and may be used as a quantitative supplement for toxicity testing. The FDA assay can be supplemented with use of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) as a probe for detection of reactive oxygen species (ROS) after toxicant exposure.

TH119

Suitability of silicon passive sampler for monitoring TBT concentrations in surface water

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Polymer based passive samplers are increasingly used in studying harmful substances in different environmental matrices. The samplers measure freely dissolved chemical concentration which is the most harmful to organisms. The samplers are deployed until the equilibrium between the sampler and the surrounding media has been reached. This enables the determination of low concentrations which in convenient grab water samples remain below the detection limit. The average concentration of studied chemical in the surrounding media can be calculated as the sampler-water partition coefficient is known. The environmental quality standard concentration (EQS) of tributyltin (TBT) in surface water is very low (0.2 ng/L) and in many cases near the laboratory detection limit. However, if TBT is not detected it doesn't mean it is not present in the environment. With passive sampling the TBT content can be enriched to the measurable level. The partition coefficient of TBT between water and a silicon passive sampler was determined in a laboratory trial by analyzing the TBT concentrations by GC/MS. As the coefficient is determined it can be used to estimate dissolved TBT concentrations in marine, surface and effluent waters. The determined partition coefficients were rather high likely due to adsorption of organotins to the sample bottle walls which decreases the freely dissolved chemical fraction C_w. The adsorbed chemical fraction is not included in our calculation since it is not available for passive samplers. The silicone passive samplers measure only freely dissolved chemical fraction so the concentrations determined with field deployed passive sampler are lower than the total ones. With silicon passive sampler the presence of TBT in aquatic environment can be revealed which further enables more reliable risk assessment.

TH120

Assessing the atmosphere-surface exchange of gaseous elemental mercury using passive air samplers

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The specific properties of gaseous elemental mercury (GEM) allow it to undergo bidirectional exchange between the atmosphere and the Earth's surface. Determining the direction and the magnitude of GEM's atmosphere-surface flux is possible and has been accomplished using micrometeorological and chamber techniques, but is (i) complex and labor intensive, and (ii) often only yields fluxes over relatively short time scales. A recently developed passive air sampler for GEM may have the precision required for identifying and quantifying vertical concentration gradients above the Earth's surface and therefore the direction and size of exchange fluxes at much longer time scales of weeks to a year and with much less effort and lower cost than existing approaches. We tested the feasibility and performance of this approach by measuring concentration gradients for one year with a monthly resolution above both natural soil in a forest and a nearby clearing, as well as above soil artificially contaminated with inorganic mercury. Significant gradients of GEM air concentrations, both increasing and decreasing with height above ground, were observed and found to be dependent on soil contamination, temperature, snow cover and solar irradiation. Because the sampler's uptake kinetics has a known, small dependence on wind speed, sampled amounts of Hg that decrease with increasing proximity to the soil surface could be an artifact caused by wind speed gradients. Measurements, however, reveal that wind differences above the soil are insufficient to explain the differences in the sampled amounts with height, implying that deposition of GEM can be recorded with this method. The method holds promise to also find use for the measurement of GEM fluxes between atmosphere and vegetation, e.g. in forest and tundra ecosystems.

New Frontiers in Life Cycle Inventory Data Collection and Modelling (P)

TH121

GeoFootprint: A publicly available web-platform with regionalised LCI data for agricultural modelling

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Life Cycle Inventory (LCI) of agricultural products (i.e. crops) tend to use the average or "archetypal" meteorological and ecological conditions for an entire country limiting the accuracy and representativeness across subregions. Specifically, the process inputs (e.g. chemical and organic fertilizers) and the accompanying release of emissions into soil, air and water (e.g. nitrate, dinitrogen monoxide, or phosphate) are sensitive to environmental parameters (precipitation, soil properties, slope, etc.) which vary spatially. Furthermore, land use change (LUC) and soil organic carbon are increasingly being recognized as major drivers of agricultural carbon footprints that are highly spatialised. Initiatives to produce regionalized LCI have not yet succeeded in developing a publicly available interface, covering multiple regions globally, available for practioner use. **GeoFootprint represents a new frontier in regionalised LCI data collection and modelling.** The initiative is led by Quantis through a grant by EIT Climate-KIC, the EU's largest public-private partnership addressing climate change through innovation. GeoFootprint aims to address the data gaps regarding spatially sensitive LCI, with focus on LUC and soil characteristics. Spatial data from Geographic Information Systems (GIS) will be merged with agronomical data (e.g. yield) in order to generate LCI that is spatially sensitive. The LCI will be democratized through a **publicly available, web-based platform by the end of 2020**. Quantis will work together with arxIT, World Resources Institute (WRI), Cool Farm Alliance and a network of leading organizations to further explore these issues and provide solutions that better support sustainable crop management practices. The network, which will be expanded over the course of the project, will provide guidance and feedback throughout the development of the platform. Initial partners include General Mills, Mars, Nespresso, Nestlé, Swiss Federal Office for Agriculture, Unilever, UPM Raflatac and Yara. This poster will **present the latest update on the GeoFootprint project** and its implications for improving regionalised LCA of agricultural products.

TH122

Challenges Associated with Life Cycle Inventory/Life Cycle Impact Assessment for Metal Mine Tailings

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The addition of human health and ecotoxicity metrics into Life Cycle Impact Assessment (LCIA) tools has increased the focus on trace amounts of metal impurities in metal mine tailings and their potential release to the environment. Recent research efforts have focused on the development of updated life cycle inventory (LCI) datasets for sulfidic tailings disposal from metal ore beneficiation. An empirical tailings model forms the basis for these LCI datasets. In this work, the development of the LCI datasets from the model was reviewed. The review identified three challenges associated with LCI/LCIA for metals released from tailings: (1) model reliability (e.g., lack of data to ground truth the LCI model, lack of a groundwater compartment in LCI/LCIA models, and limited consideration tailing management practices); (2) data representativeness (e.g., potential for inconsistent extraction protocols, lack of paired composition/leachate data, nature of operation/facility) and (3) uncertainty (e.g, definition of acceptable levels of uncertainty). The following actions/recommendations address some of these challenges: (1) Available geochemical models for tailings are being evaluated for use as a resource to validate/refine the LCI tailings model formulation. (2) A guidance document discussing data representativeness guidelines for tailing composition/leachate chemistry dataset inclusion in the model database should be developed to ensure data quality. (3) Efforts are being undertaken to bolster the tailings composition and leachate chemistry database. (4) The effect of applying the Best Available Techniques (BATs) for tailings management should be assessed as an additional scenario at a minimum. The long-term performance of the BATs is worth considering in more detail. (5) Attenuation of leachate metals in groundwater is expected but must be quantified. In the long-term, a groundwater compartment—specifically its impact on leachate metal fate--should be included in the USEtox LCIA model.

TH123

Metal emission quantification, relevant aspects from an LCA perspective

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Agricultural production contributes significantly to several impact categories such as eutrophication, acidification or toxicity. Direct farm emissions strongly depend on farm management practices and are influenced by location characteristics. In the last decade, life cycle assessment (LCA) has been increasingly improved and used for the quantification of these category indicators. However, an aspect that still needs a deep understanding is the quantification of metal emissions from agricultural systems. Currently, simplistic approaches to model metal emission fractions into the different environmental compartments are implemented, from which the most widely used is the SALCA methodology. Several shortcomings in this approach have been identified. Therefore, the aim of this study is to advance and agree on the methodology to improve the current quantification of metal emissions from agriculture and its harmonization with characterization models. As a first step, a literature review was performed to give an overview of the relevant metal sources of emission from different agricultural activities. Inventory is built following the methodological guidelines for agricultural products, in a balance of metal inputs and outputs. We have considered seven metals from agricultural sources: cadmium, chromium, copper, lead, nickel, and zinc. The different sources of emission were classified into four categories: synthetic fertilizers, organic-related fertilizers (e.g., bio-solids, manure, compost, etc.), pesticides (as dissolved metal forms such as copper-based fungicides) and wastewater irrigation. Regarding the mass balance between inputs and outputs, we suggest improvements by adjusting the inputs on the balance for different application inventories, re-calibrating the model by using experimental data for specific soil characteristics; and by updating the metal releases from the different emission sources. Furthermore, a better discrimination for the bioavailable fraction of metal by taking advantage of the knowledge of biological tests and biological sensors is accounted. These findings and recommendations also demonstrate the need and relevance of spatially differentiated inventory of metal emissions that are harmonized and can be used with characterization models for life cycle impact assessment to support effective decision-making and finally a consensus agreement similar to that for pesticides assessment would be advisable.

New Developments in the Science of vPvB and PBT Assessment (P)

TH124

Initiative towards improving our understanding of persistence in the 21st century

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There is increasing awareness that progress in the scientific understanding of factors which influence the outcome of persistence (P) assessments of chemicals (particularly biodegradation) are not fully recognised in existing regulations and environmental risk assessments. As all biodegradation tests have their limitations, which are accentuated for 'difficult to test' substances, failure to recognise these can potentially lead to chemicals being incorrectly labelled as 'P', 'vP' or not P. Many of the shortcomings of existing biodegradation studies have been previously recognised and discussed in a series of ECETOC reports and workshops which were subsequently used to help develop and formulate a series of research projects to try and address key issues and propose methods to mitigate the existing limitations of persistence assessments. However, many of the recent developments recommended in the multi stakeholder workshops and related research projects have not been incorporated into existing regulatory frameworks for environmental hazard and risk assessment. Due to growing awareness that this situation needs to be rectified a Persistence Workshop was held in Helsinki on the 27th September 2018. The objective of the workshop was to disseminate key findings from recent projects and develop proposals regarding how the current situation can be improved to ensure appropriate classification of the hazards posed by a wide range of chemicals. The challenge being to develop consensus regarding how recent knowledge can be incorporated into existing regulatory frameworks to improve the environmental hazard and risk assessment of chemicals. From the outset it was recognised that any proposal to modify guidance needs to be based on sound science, transparent and relevant to all stakeholders. The workshop provided a unique opportunity to initiate a process to re-examine the fundamentals of biodegradation and what existing test methods can achieve by 1) providing an overview of some of the key elements and messages coming from recent research initiatives and 2) stimulating discussion regarding how these interrelate and can be used to redress some of the previously identified persistence assessment needs. This presentation will provide a summary of the key messages from the workshop and subsequent progress on the next steps required to start a journey to take the persistence assessment of chemicals into the 21st Century.

TH125

Relationship between biodegradation and bioaccumulation. Practical outcomes.

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According to the REACH Regulation, for all substances manufactured or imported in amounts of 10 or more tons per year, that are not exempted from the registration requirement, a Chemical Safety Assessment (CSA) must be conducted. According to CSA criteria, for these substances a PBT/vPvB assessment is requested. PBT/vPvB properties are defined by the criteria described in Annex XIII to the REACH Regulation. If based on the available information the substance is potentially B/vB and fulfils the P/vP criteria, experimental data according to the OECD TG 305 protocol should be generated. In order to reduce the number of applications of the expensive bioaccumulation test it seems useful to search thresholds for other related parameters below which no bioaccumulation is observed. Taking into account the known relationship between ready biodegradability and bioaccumulation such parameter is biodegradation. Given the stringent regulatory criteria for avoiding *BCF* tests based on *BOD* it is interesting to investigate in more details this relationship in searching for *BOD* threshold below which practically no vB/B chemicals could be observed. It is especially interesting to relate the *BOD* criteria for not vB/B chemicals with regulatory criteria for persistency which are in the range of 10% - 30% *BOD* depending on local regulations determined in terms of half-life. This analysis is the first aim of the present work. The second aim is the analysis of the relationship between the rates of *in vivo* metabolic transformations and bioaccumulation thresholds. Metabolic transformation rates were defined ensuring low bioaccumulation of chemicals, in terms of vB and B thresholds.

TH126

Membrane partitioning of organic cations, anions and ion pairs - estimating the bioconcentration potential of charged compounds

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Experimental measurements of bioconcentration factors (BCFs) are not feasible for large sets of chemicals. In the absence of experimental data BCFs are often predicted using quantitative structure activity relationships (QSARs) employing octanol-water partition coefficient (log Kow) as a descriptor. This approach often underestimates BCFs of charged speciesbecause it neither sufficiently considers the interactions of charged compounds with the heterogeneous membrane system that contains, e.g., polar and charged phospholipids nor interactions of cations and anions in solution – both strongly influencing the transport, uptake and bioavailability of ions. The aim of this study was to use log K_{MW} to obtain a more realistic estimation of the bioconcentration potential of organic cations and anions because lipid membrane-water partitioning coefficients (log K_{MW}) generally correlate better with BCF than the log Kow. The interactions of organic ions and ion pairs with biological membrane lipids and their consequences in terms of bioconcentration were investigated in order to develop QSAR that can be used in estimation of bioconcentration potential of permanently charged species. The log K_{MW} values showed that tested cations and anions can have a strong affinity for phosphatidylcholine bilayers. The results need to be verified *in vivo* but they indicate considerable bioaccumulation potential. The log K_{MW} values for the organic cations were orders of magnitude higher than log K_{OW} values reported in the literature, suggesting that log K_{OW} might not be a valid descriptor for this group of compounds. Moreover, the partitioning of organic ions is expected to be more dependent on environmental parameters than what is known for neutral compounds. Our study indicates that interactions with other ions in particular need to be considered when assessing the bioconcentration potential of hydrophobic cations and anions, including the type of counter ion, the concentration and type of the other hydrophobic ions in the medium (other pollutants) and the ionic strength of the medium (e.g., fresh vs. marine water)

TH127

Assessing Persistence, Bioaccumulation and Toxicity (PBT) properties of Unknown or Variable Composition, Complex Reaction Products and Biological Material (UVCB) hydrocarbon solvents

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Under European Union's Registration, Evaluation and Authorization of Chemicals (REACH) Regulation, assessing PBT properties is required for all chemicals for which a chemical safety assessment is required. Many petrochemicals, including hydrocarbon solvents, are UVCBs and thus the assessment can be challenging as the current capabilities of analytical chemistry precludes the resolution of every chemical present in a substance. This is of particular concern to regulators as the ECHA REACH Guidance Chapter R.11 PBT/vPvB assessment (2017) states that any constituent > 0.1% should be evaluated for PBT properties. However, when the carbon range is sufficiently narrow, whole substance testing should be employed as a method to evaluate PBT properties. Here we demonstrate a weight of evidence approach to evaluating PBT properties of hydrocarbon solvents. This involves comparisons of laboratory whole substance biodegradation tests (e.g. OECD 301F manometric respirometry) along with biodegradation data on individual constituents that demonstrate that the whole substance will not persist in the environment. Water accommodated fraction testing demonstrates the biologically available fraction does not induce toxicity, and thus are not T. Finally, we present new biomagnification (BMF) on two solvents demonstrating a method for evaluating bioaccumulation potential and demonstrating the substances do not biomagnify. The evaluation of hydrocarbon solvent UVCBs is challenged by technical limitations in resolving every individual constituent. As such, a weight of evidence approach towards PBT assessments should be adopted, using whole substance laboratory data when available. In this work, we show that when a weight of evidence approach is applied, the hydrocarbon solvents do not meet the criteria for PBT.

TH128

Solving complexity of biodegradation assessments for UVCB chemicals using an automated system: EVABIO

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According to the United Nations (UN), a substance is the "chemical elements and their compounds in the natural state or obtained by any production process". This definition has evolved according to different acts of regulation. Now days, thousands of new chemicals require a biodegradability assessment annually. For this task, the development of new metrological solutions needs to be investigated to achieve our assessments needs, especially for complex chemicals, such as UVCB*, for which measurement systems are lacking. For UVCB, assessing biodegradability can be limited by technical issues and/or difficulties to rule on ready or inherent biodegradability. To increase reliability of this assessment, our objective was to develop a multiparametric platform disposing of a global measuring methodology based on both O_2 consumption and CO_2 releasing. Different technologies of sensors have been validated in operating conditions to establish a carbon balance measurement, using several modeling steps involving the use of different parameters such as O_2, CO_2, pH, T°C, Pressure and Biomass. Finalization of these investigations enabled the development of an automated device dedicated to biodegradation assessment of chemicals, whatever their complexity (pure chemicals to UVCB). The multiparametric platform will discriminate inorganic carbon released and organic carbon allocated in biomass. According to the UTOC methodology developed to highlight UVCB fate (Ultimately Transformed Organic Carbon), final conclusion onto UVCB biodegradability will be done in a weight of evidence analysis including both results of biodegradation and ecotoxicological potential of biodegradation by-products (Brillet et al., 2018). **Keywords:** Biodegradation assessment, biodegradation platform, UVCB, complex chemicals, biodegradability * UVCB stands for Unknown or Variable composition, Complex reaction products or of Biological materials.

TH129

Investigating the effect of test concentration on biodegradation kinetics of two hydrophobic UVCBs

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While risk assessment of mono-constituent substances has for many years been in focus, regulatory and scientific attention has currently shifted towards investigating the exposure, fate and effects of chemical mixtures. This includes multi-constituent substances and substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCBs). Petroleum and essential oils are UVCBs, and difficult to test as they cannot be described by a simple chemical structure and contain unknown constituents. In addition, they contain highly hydrophobic organic chemicals. In this study we combined a recently established platform for biodegradation testing of hydrophobic and volatile chemicals with new advancements in passive dosing to determine biodegradation kinetics of two complex and hydrophobic mixtures, diesel oil and lavender oil. The aims were to 1) investigate the effect of changing the initial mixture concentration on the biodegradation kinetics and 2) to couple delayed biodegradation to toxicity, measured as inhibition of ^{3}H-leucine incorporation. Passive dosing from a silicone rod was used to set mixture composition and mixture concentration level in the tests and facilitated stable and reproducible initial test concentrations in many replicate test systems. All tests were carried out in gas-tight autosampler vials with a headspace. Automated head-space Solid Phase Microextraction (HS-SPME) was carried out directly on the test systems and coupled to gas chromatography mass spectrometry (GC-MS) for chemical analysis. The first results show a correlation between test concentration, biodegradation kinetics and toxicity and provide a basis for further studies on biodegradation of complex mixtures.

TH130

Changes in sediment bacterial community diversity and structure throughout an OECD308 test with ten micropollutants

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The OECD308 standard test is widely used to assess the degradability of pollutants in water-sediment systems, however, the recommended settings for the test often do not reflect the original conditions in the natural environment. In this study, we hypothesized that bacterial communities present in the sediment, which are responsible for biotransformation of pollutants, might shift due to differences between environmental and test conditions. We performed OECD308 bottle incubations using sediment from two rivers (F and G), before (1) and after (2) the discharge of a local waste water treatment plant, after 10 day acclimation period, triplicate-sets were spiked with a mix of ten micropollutants at two concentration levels (20ug/l and 2000ug/l) and incubated for 40 days. An extra triplicate control was not spiked with micropollutants. Bacterial community composition was analysed using Illumina sequencing of bacterial 16S rRNA in sediment samples collected directly from the rivers, samples after the acclimation period, and after the incubation period. The bacterial community structure was generally found to be significantly different between rivers and sites ($p\text{-value} \leq 0.001$). Bacterial diversity did not show consistent behavior along the test, and generally remained stable. However, the specific composition of the bacteria community was variable throughout the study. The most significant changes in the bacterial communities appeared between the river sediment collection and the end of the acclimation period, where i.e. bacteria of the Burkholderiales and Rhodospirillales orders had a significant decrease in abundance. Interestingly, in our study we associated several bacteria genera belonging to these orders to fast degradation of micropollutants. Significant changes were also observed between the acclimation period and the end of the test for bacteria in four orders. No significant differences were found in the bacteria community composition between the two concentration levels and the non-spiked control, showing bacteria communities can be robust against high concentrations of micropollutants. Our results show that conditions used for the OECD308 test can affect the bacterial community structure, thus the degradation observed in OECD308 may not necessarily represent degradation observed in the environment and that the implications for risk assessment should be evaluated.

TH131

Comparing chemical persistence assessed using unspiked OECD 309 test to field measurement

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Biodegradation is a primary removal pathway thus a major persistence determinant for many organic contaminants in natural systems. The OECD 309 guideline is important for providing biodegradation kinetic data in surface waters for use in persistence and risk assessments. According to the OECD 309 protocol, biodegradation is measured in aerobic natural waters spiked with test chemicals and incubated in the laboratory. However, results from our prior study with an unspiked OECD 309 test combining non-target analytical approaches confirmed that spiking natural aquatic systems with test chemicals can strongly influence biodegradation measurements, most likely due to the adaption of microbial organisms. This study was designed to further evaluate the environmental relevance of the unspiked OECD 309 test by comparing chemical biodegradation half-lives assessed in the laboratory against their persistence directly measured in the field by a benchmarking approach. To this end, Lake Norra Bergundasjön in southern Sweden was selected, a recipient for wastewater treatment plant (WWTP) effluent with a freshwater dilution factor of ~4. For measuring chemical persistence in the field, the WWTP effluent discharged to the lake as well as surface water of the lake inlet and outlet were collected over a period of 3 months in 2017. The samples were analyzed with a weekly temporal resolution to verify that chemicals in the lake were in steady state. The conservative chemical bicalutamide, which was detected constantly throughout the whole sampling period, was used as the benchmark chemical. For assessing chemical biodegradation in the laboratory, an unspiked OECD 309 test was carried out in duplicate using fresh lake water collected at the end of the field sampling period. The test was under conditions recommended by the standard OECD 309 protocol and lasted for a period of 60 days at 20 °C in the dark. Triplicated water samples were collected at 11 time points. All samples were filtered and analyzed with UHPLC-Orbitrap-MS/MS using a direct injection method. Data was processed using a non-target approach. Biodegradation half-lives of organic contaminants detected both in the laboratory test and in the field will be calculated and contrasted.

TH132

Compartment-Specific Screening Tools for Persistence - Potential Role and Application under REACH

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The persistence assessment under REACH relies on compartment-specific degradation half-lives derived from laboratory simulation studies with surface water, aquatic sediment or soil. Although these data are given priority, they are not available for most of the compounds. Thus, in a first step according to the current Integrated Assessment and Testing Strategy (ITS) for persistence assessment in REACH guidance R.11, a substance is generally considered to be not persistent, if it is readily biodegradable in water and there is no need to perform further biodegradation tests for the PBT assessment. Ready biodegradability tests (RBTs) must be designed so that positive results are unequivocal, i.e. rapid and ultimate biodegradation in the environment can be assumed. However, the fate of a substance may differ substantially between aquatic systems and soils or sediment, which are very complex and heterogeneous matrices with a large amount and variety of binding sites. This might result in sorption and a reduced biodegradability. Nevertheless, soil and sediment compartments are still not considered in existing RBTs and simulation studies covering all relevant environmental compartments (i.e. water, aquatic sediment, soil) are only required if the substance cannot be rated as non-persistent based on all available information in step 4 of the ITS. Consequently, there is a lack of experimental methods suitable to derive biodegradation data in soil and sediment at the screening test level, which can be used to identify potential persistence in sediment or soil. In previous studies, compartment-specific screening tools for water-sediment (Water-Sediment Screening Tool; WSST) and soil (Soil Screening Tool; SST) have been developed and were successfully applied to determine sound and reliable biodegradation data for a set of test compounds. The results were used within a new alternative persistence screening approach, the Compartment-Specific Persistence Screening (CSPS). In this poster, the CSPS is presented and the results are compared with the outcome of the REACH standard persistence screening. The differences between the two approaches are discussed and potential scopes of application for the WSST and the SST in the context of the REACH ITS for persistence assessment are proposed.

TH133

Persistence assessment of Plant Protection Products (PPPs) in the light of a changing EU regulatory environment

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Persistence (P) assessment is hazard based and performed in the framework of a Persistence Organic Pollutants (POP; UN level) and PBT (Persistent, Bioaccumulative, Toxic) / vPvB (very Persistent, very Bioaccumulative) (EU level) classification. To assess potential PBT / vPvB properties, the criteria were introduced into different, substance-specific EU legislations for industrial chemicals (EC No. 1907/2006), Plant Protection Products (PPPs; EC No. 1107/2009) and biocidal products (EC No. 528/2012). PBT guidances implemented in these different regulations specify the same trigger values for assessing P and vP, but different criteria on how to derive trigger endpoints are applied; this is in particular true since the latest REACH PBT guidance update in 2017. In addition, also the consequences of a PBT / vPvB classification are differing. While a PBT / vPvB classification is a direct cut-off for PPPs, mitigation, restrictions or further registration requirements are applicable for industrial chemicals. These differences are particularly critical due to the observed tendency towards a harmonization of the PBT / vPvB assessment for the different chemical classes. Although the DG SANCO PBT guidance from 2012 is applicable for PPPs (noted by the Standing Committee on Plants, Animals, Food and Feed [SCoPAFF]), applicants for PPP authorizations experienced of late that the REACH PBT guidance is more and more used by the European Food Safety Authority (EFSA) and Member States (MS) authorities. For example, applicants received requests for soil and water half-life (DT_{50}) values normalized to 12°C, although the SANCO PBT guidance specifies a reference temperature of 20°C for assessing P. As a consequence, a 12°C normalization would lead to significant increase in the number of PPPs classified as "P" when applying the currently valid trigger values which, in turn, would lead to a disablement of this hazard tool. Logically, when normalizing DT_{50} values to a specific reference temperature, the currently applicable trigger values, which were orginally derived at room temperature, should be normalized to the identical temperature. Overall, these developments result in a high degree of uncertainty, especially for the applicants of PPPs (but also for MS). There is no clarity for the applicant on how a P assessment should be performed presently and in the future. Therefore, EFSA is requested to provide guidance on that topic, involving all stakeholders in this field.

TH134

Temperature dependency of biodegradation kinetics in environmental surface waters

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Biodegradation kinetic data are keystone in persistency assessments and risk assessments of chemicals. Biodegradation is temperature dependent, and biodegradation tests used for persistency assessments should be conducted at standard test temperatures, which were recently lowered from 20°C to 12°C in the Guidance on Information Requirements and Chemical Safety Assessment from ECHA (2017). Current practice in compensating for the difference in standard test and environmental temperature is to assume that microbial communities have the same temperature response and uniform biodegradation capacity at a given temperature[1]. These assumptions have led to the use of a "rule of thumbs" correction factor based on the Arrhenius equation for extrapolation of biodegradation rates from one temperature to another. This practice is however discussed as it is based on abiotic, not biotic degradation rates. It was recently reported that the temperature effect differed, for example, amongst compounds depending on their chemical structure[2]. Comparative biodegradation kinetic data at different test temperatures are needed for a large number of chemicals in order to improve extrapolation between temperatures in risk assessment and predictive modelling. Here we present an experimental setup that will provide biodegradation kinetics for approximately 40 petroleum hydrocarbons (HCs) at origin of inoculum temperature and two standard test temperatures for three different surface waters. Natural inoculum is sampled from Northern, Middle and Southern European Rivers to cover a broad temperature range. The HCs are tested in composed mixtures at environmental realistic low concentrations generated by passive dosing, and the tests performed in air-tight vials are incubated at two different temperatures. After incubations of 0-28 days, triplicate test systems are analyzed by Headspace Solid Phase Microextraction (HS-SPME) Gas Chromatography Mass Spectrometry (GC/MS), and the concentration relative to abiotic controls are used to model biodegradation kinetics[3]. The obtained dataset can also be compared with literature. From the Northern European site, the biodegradation will additionally be determined using three different samples to cover seasonal variance. [1]Bagi, A. *et al.* (2013) *Mar Environ Res*, pp. 83–90. [2]Birch, H., Hammershøj, R. and Mayer, P. (2018) *Environ Sci Technol*, pp. 2143–2151. [3]Vergeynst, L. *et al.* (2018) *JHazard Mater*. Elsevier, pp. 127–134.

TH135

Challenges and Experimental Issue for Kinetic Simulation Studies conducted at 12°C (OECD TG 309)

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Historically most kinetic simulation studies were conducted at a standard laboratory temperature of 20 +/- 2°C. With the update of ECHA IR and CSA Guidance r.7b and R.11 new kinetic simulation studies as required for persistency assessment of industrial chemicals must be conducted at environmentally relevant temperatures. For the European Union 12°C are regarded as a reasonable average temperature for fresh water systems. The OECD TG 309 as a prelagic test at 12°C is the preferred study to be carried out independing of the physical-chemical properties and the exposure pathway of the substance. Most data for physical-chemical properties (e.g. water solubility) are generated at a temperature of 20°C in pure water and so far only very limited data for solubility at 12°C in natural surface water are available. Especially for substances with low water solubility testing is a challenge and the set-up of a reliable experimental test design becomes an issue. Another aspect is that due to the slower decline the uncertainties in deriving half-lives by kinetic fitting are increasing. Acc. to FOCUS guidance the data must be of sufficient quality to clearly establish the dissipation pattern. At 12°C the degradation is slower and a decline to 50% of the initial concentration might not be reached within the standard study duration of 60 days. Experiences with studies conducted at 12°C will be presented.

TH136

Aerobic Transformation of a Biocide in an Aquatic Marine Sediment System at 9°C

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A modified OECD 308 study was performed at 9°C to determine the amount and rate of degradation of a biocide at the more environmentally relevant temperature of 9°C than the OECD 308 guideline temperature of 20°C. This study had the following adaptations and enhancements from a standard OECD 308 to establish if degradation was improved: The temperature assessed was lowered to 9 ± 2°C Test substance was radiolabelled in two positions to enable tracking of different metabolites The seawater had mineral media added (following the OECD TG 306) Vessels were dosed either into the water phase or the sediment phase to monitor any differences in degradation Sediment was not sieved to ensure optimal viability at study initiation Lower water:sediment ratio (1:1.6) to minimise movement of test substance between water and sediment Non-extractable residue (NER) analysis was performed on all sediment samples to determine if the residues were Type I NER (sequestered, entrapped NER), Type II NER (covalently bound NER) or Type III NER (biogenic NER). **Keywords**: Biocide; marine sediment degradation; Non-extractable residue (NER); OECD 308

TH137

How to characterise non-extractable residues (NER) in PBT assessment - development of a harmonised procedure to be used in routine testing

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In degradation testing in soils and water/sediment systems for regulatory purposes according to OECD standard guidelines, very often a fraction of the test substance is observed, that cannot be released from the solid matrix with non-destructive extraction methods. These so called Non-Extractable Residues (NER) can be detected only, if isotope labelled test substance is used, which is mandatory in regulatory framework if technically feasible. The importance of NER in the persistency assessment has been more or less neglected in the past. But according to new developments e.g. in REACH guideline revisions (ECHA R.11, 2017) the NER has to be considered as they may potentially be remobilised as parent or transformation product. One approach is to consider NER as 100% potentially available parent substance if not proven otherwise. This worst case assumption might be misleading because NER can also represent residues or products of degradation without any environmental relevance as they are irreversibly bound or transformed into biomass. Therefore, the ECHA published in June 2018 the discussion paper "*Consultancy services to support ECHA in improving the interpretation of Non-Extractable Residues (NER) in degradation assessment*" where guidance is given on how to characterise NER and the different NER types in practical testing. However, the discussion paper clearly states that discussions on NER assessment are still ongoing and the current paper represents a state of the art report only. In parallel and on behalf of the German Environment Agency (UBA) a project was conducted in order to develop a straightforward extraction procedure for NER characterisation for use in practical testing (FKZ 3713 63 413 1). The project results differ to some extent from the procedures described in the discussion paper and the question came up, how an acceptable method for use in regulatory routine testing can be derived. Thus, in September 2018 the German Environment Agency (UBA) initiated a project (FKZ 3718 65 407 0) in order to develop a harmonised procedure for practical testing taking into account both current approaches and proving the applicability of the harmonised approach experimentally using a set of reference substances. The project results will be discussed with regulators, scientists and stakeholders in an international workshop at the end of the project and it is intended to set the standard for future consideration of NER in chemicals persistency assessment.

TH138

Part II: Non-extractable residues (NER) in soil - A regulatory view.

T. Junge, knoell Germany GmbH

In order to assess the environmental fate of chemicals knowledge on their biotic and abiotic degradation is essential and is a key factor in risk assessment and their registration. While for industrial chemicals the main focus lies on screening tests to assess biodegradation, extensive degradation tests are needed to investigate the route and rate of degradation of plant protection products (PPP). This poster part II is complemented by poster part I outlined in a separate abstract (*F. Schnitzler et al., Part I: Non-extractable residues in soil – Review and definitions*). In general, degradation of chemicals in soil results in the formation of degradation products (i.e. metabolites), non-extractable residues (NER), microbial biomass and finally in their mineralization. Whereas on the one hand degradation products and as a final step mineralization (i.e. formation of CO_2) are rather easy to identify, assess and evaluate in laboratory tests on the other hand composition, nature and identity of NER are often unknown. As a consequence of these uncertainties, the question arises when NER should be considered as a safe sink and when as a potential risk. Beside the potential bioavailability and bioaccessibility of NER a major concern is a possible slow release from soil over the long term. Therefore, NER are an important parameter not only in risk assessment (i.e. PEC/PNEC) but also in hazard/persistence assessment (PBT) of chemicals. Regarding EU regulatory aspects of NER there are generally two essentially different views for authorisation of industrial chemicals and PPP. Within the regulation of industrial chemicals a precautionary approach is followed considering NER as non-degraded parent which generally overestimates the exposure concentration. For PPP a less conservative approach underlies their regulation considering NER as degraded residues of the active substance which are of no further concern representing a safe sink. However, a general cut-off criterion is laid down for PPP regarding the maximum amount of NER formed in combination with a low mineralization rate. On the basis of current regulations regarding NER in soil their evaluation in environmental risk assessment and PBT assessment within the regulatory context will be presented and discussed.

TH139

Part I: Non-extractable residues (NER) in soil - Review and definitions

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This poster aims to provide an introduction to the topic and goes back to the roots of the non-extractable residues (NER) concept in presenting an overview of the history, the evolvement of definitions and naming conventions over time and the current scientific state of the art. This poster part I is complemented by poster part II outlined in a separate abstract (*T. Junge, Part II: Non-extractable residues in soil - A regulatory view*). Based on different types of binding several types of NER are defined which correlate with the binding strength of (agro-)chemical compounds to soil. To characterise these bindings a wide range of extraction methods is commonly used. Advantages and disadvantages of these applied methods from gentle (i.e. shaking at ambient temperature) to harsh (i.e. supercritical fluid extraction) are presented and discussed. The formation of NER in soil is closely linked to the (bio)availability of (agro-)chemical compounds and their degradation kinetics. Implications from the formation of different types of NER on persistence and/or toxicity are briefly highlighted in a regulatory context before being discussed in more depth on poster part II. A scientific state of the art overview is provided regarding the characterisation and/or identification of xenobiotic NER and biogenic NER as well as the determination of (bio)availability. In parallel, also areas where sufficient insights are still missing today are presented: e.g. the lack of knowledge about the chemical nature of NER and how to detect NER with reliable methods and tools (experimental or modelling) to evaluate NER toxicity. Although NER represent a topic that has been discussed for decades it is still far from completion and leaves the door wide open for new scientific findings and regulatory developments.

TH140

Bioaccumulation assessment: new methods need new testing and assessment strategies - proposal of an ITS

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Bioaccumulation assessment, usually mainly relying on the OECD test guideline 305 (the fish flow-through test) is part of the hazard and risk assessment in the context of registration and authorisation of industrial chemicals, pesticides, biocides etc. A number of alternative testing methods have been and are being developed, such as the in-vitro test guidelines for the determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes and rainbow trout liver S9 sub-cellular fraction (OECD TG 319 A and B) and the new BCF test using *Hyalella azteca.* Both, standardised and non-standardised tests will need to be integrated into a coherent testing and assessment strategy including also other available tests such as OECD TG 315 and 317 covering bioaccumulation testing in terrestrial and benthic oligochaetes. Aspects which need to be considered are specific regulatory requirements, e.g. in cosmetics assessment, tests with vertebrates cannot be used. Also, substance specific testing conditions need to be addressed, as required for compounds such as surfactants, superhydrophobic or ionisable chemicals. The practical requirements for bioaccumulation testing of nanomaterials are still not clearly defined and adjustments of the established test systems are mandatory. The poster will discuss a possible integrated testing strategy (ITS), considering the different applicability domains of the tests and regulatory needs.

TH141

Bioconcentration of laurate in the freshwater amphipod Hyalella azteca

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Regulatory assessment of bioaccumulation from water is commonly based on bioconcentration factors (BCF) derived from fish flow-through tests (OECD 305). Such experiments require a lot of laboratory animals and are time-consuming and costly. Further, the 3R principle postulates the replacement, reduction and refinement of vertebrate tests. An alternative test set-up for organic, neutral compounds using the freshwater amphipod *Hyalella azteca* was suggested. However, there is a lack of information on the bioaccumulation of ionic compounds in *H. azteca*. In this study the bioconcentration of the lauric acid anion laurate in *H. azteca* was investigated to elucidate the bioaccumulation potential of the test item in comparison to published data on zebrafish. In a first study, radiolabeled 1-^{14}C sodium laurate was applied to *H. azteca* in a flow through system. Media samples were analysed via LSC and radio TLC. Tissue concentrations were determined via LSC after combustion. Laurate showed to be rapidly biodegradable. Therefore, a second BCF-test was carried out under semi-static conditions to ensure stable exposure concentrations. Methods for tissue analysis were adjusted to allow an accurate quantification of the test item. Laurate was incorporated into various lipid fractions of *H. azteca*. However, more than 60 % of the total radioactivity found in the amphipod tissue was not extractable but could partially be driven out by acidification and captured as CO_2. The results suggest that also carbonate (CO_3^-) resulting from the mineralisation of laurate was bioaccumulated in the calcified exoskeleton of *Hyalella azteca* consequentlyleading to an overestimation of tissue concentrations,if accumulated compound was simply measured as total radioactivity and not further fractionated. The calculated BCF estimates differ from the one described in the literature for zebrafish. The results show that bioaccumulation of laurate varies between *H. azteca* and zebrafish. The mechanisms involved in the bioconcentration of the anionic compound seem to be more complex and the results cannot be extrapolated to other species without further research. Special care is required for *Hyalella* bioaccumulation studies with ^{14}C-labelled compounds which are rapidly mineralised.

TH142

How do fish bioconcentration factors for nonionic organic substances derived using dietary exposure compare with aqueous tests?

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Traditional approaches to characterizing bioaccumulation based on aqueous exposure bioconcentration factors (BCFs) are often challenging for "difficult to test substances". Particularly, substances that have low solubility, or readily volatilize or degrade make it challenging to maintain constant exposures during aqueous BCF tests. An alternative dosing method, via dietary exposure offers several key advantages for these substances. Dietary exposures provide simpler, more consistent, and more relevant dosing methods for poorly soluble, difficult to test, substances as well as reduce the number of test organisms required. Further, dietary exposure and use of the resulting biomagnification factors (BMFs) allow the mitigating role of both tissue and gastrointestinal biotransformation to be quantified. Finally, the use of dietary exposures enable the BMFs (and consequently BMF-derived BCFs) to be derived using a mass-balance model framework. The purpose of this study is to compare BCFs derived from dietary BMFs with independently-determined BCFs from aqueous exposure tests, for a range of substances. These dietary-derived BCFs were compared to the aqueous BCFs to determine if these two exposure pathways are in agreement and to determine if dietary BMFs can be reliably used to estimate aqueous BCFs, which are currently used as the primary metric for "B" criteria in PBT assessment. This work has implications for applying existing and new dietary BMF data in bioaccumulation assessment frameworks.

TH143

How to determine KOA in the bioaccumulation assessment of air-breathing organisms?

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Respiration is a contaminant elimination pathway in air-breathing organisms, that can prevent bioaccumulation of sufficiently volatile substances. In particular, a substance with an equilibrium partition coefficient between air and octanol K_{OA} at 25 °C below 10,000 may be regarded as non-bioaccumulative, because it is rapidly eliminated with exhaled air. Here we explore how this threshold could be employed in a first tier of a screening process for identifying chemicals that are potentially bioaccumulative in air-breathing organisms. Specifically, we are evaluating the performance of different K_{OA}prediction and measurement techniques within the threshold range of $3 < \log K_{OA} < 7$. This range is selected assuming that substances with a predicted $\log K_{OA} < 3$ are clearly too volatile to bioaccumulate, whereas those with a predicted $\log K_{OA} > 7$ are clearly not sufficiently volatile for exhalation to be an effective elimination pathway. Different K_{OA}prediction techniques, including various QSPRs, poly-parameter linear free energy relationships, and methods based on quantum-chemical predictions and statistical thermodynamics, are evaluated vis-à-vis a database of measured K_{OA}values reported in the literature. Based on the predictions of the methods found to perform best in the threshold range, a decision is made whether an experimental determination of the $K_{OA}$is required ("Is the predicted K_{OA} in the threshold range?"), and which technique is preferred ("Is the predicted K_{OA} at the upper or lower end of the threshold range?"). Experimental techniques relying on the analysis of the gas phase in closed vials equilibrating an octanol phase with the overlying headspace are most suitable for measuring K_{OA} at the lower end of the threshold range, whereas dynamic generator column techniques, that equilibrate a gas flow with a stationary octanol solution, are preferable for those at the higher end of that range.

Towards a Science-based Risk Assessment Framework for Nano- and Microplastic (P)

TH144

Spatiotemporal Trends of Microplastics Loading in the Sediments of River Ganga: First Observation on Occurrence, Identification and Quantification

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Microplastics (plastics less than 5 mm) with their ubiquitous presence and distribution is a newly recognized potential threat to aquatic ecosystems because of their direct toxicity to organisms or from the toxic pollutants they transport. However, the majority of the research has focused on microplastic load and toxic effect on marine ecosystems rather than on the freshwater aquatic ecosystems that are more closely connected to terrestrial microplastic contamination sources. River Ganga is the largest river in India and is a lifeline for millions of people as well as aquatic flora and fauna. However, excessive use of plastics in various sectors complemented with the scarcity of land for waste disposal and poorly developed waste management infrastructure makes River Ganga an uncontrolled dumping ground for plastics load, that may eventually put the health of its aquatic system into jeopardy. To best manage this microplastic contamination and minimize their exposure to aquatic organisms in River Ganga, it becomes highly imperative to develop comprehensive risk assessment and mitigation strategies. A well-defined monitoring strategy and quantification of microplastics contamination load, both spatially and temporally, will help to identify at-high risk zones, and thereafter accordingly develop effective monitoring and mitigation strategies. This study attempts for the first time, the spatiotemporal variation in the microplastic contamination of sediments from entire river Ganga as it passes through five Indian states starting from the rocky Himalayas to the heavily populated and industrialized plain lands. Sediments samples were collected from 55 sampling locations across the entire 2525 Km stretch of river Ganga through two seasons viz post monsoon and post-winter (2017-2018). Microplastics were extracted from these sediments with an optimized extraction protocol. Further, identification and characterization of the polymer types is performed using μ-FTIR. The results of the study provide a first time account on occurrence and levels of microplastic contamination for arguably the most sacred river of the world, i.e. river Ganga, which will help decision makers in policy formulation for managing pollution sources and combating their distribution. **Key words:** Ganga; Microplastic; Sediment; FTIR

TH145

Modelling emissions of microplastics in Europe from wastewater sources, including land applied biosolids

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Public information regarding microplastics in the environment is frequently available and comes from a variety of sources, often in the form of retrospective sources such as measured aquatic data. Science-based risk assessment must utilize both retrospective and prospective exposure information to effectively estimate potential risk to ecological receptors. While monitoring data provide information at only a few locations for several points in time, prospective models can estimate the potential for ecological exposures across many landscapes and over long periods of time, and both have a role in risk assessment. Wastewater treatment plants are often cited as a source of microplastics entering the environment. Microplastics are highly removed (generally >90%) during the waste water treatment process, via skimming of floating particles or sorption to solids and settling into sludge. Understanding the eventual fate of this sludge, and the potential for contained microplastics to re-enter surface water, is one step of many in determining the fate of microplastics in the aquatic environment. Sludge management in Europe varies geographically, with up to 90% of sludge used on agriculture in Portugal, and 0% in other countries (Eurostat, 2017) with other disposal including incineration, landfill or composting. We present a model which addresses both direct aquatic emissions into surface water via waste water effluent, as well as indirectly from land applied biosolids coupled with spatially-defined surface runoff potential. Generalized runoff potential is estimated using fate and transport models used for plant protection products found in the EFSA FOCUS scenarios. To our knowledge, this coupling of direct aquatic emission and sludge-biosolids-runoff is a novel approach for examining environmental emissions of microplastics which enter municipal wastewater treatment plants. This spatially-explicit model is based on publicly available datasets, combined with a hydrologic framework containing geographically variable emissions linked to a river network simulating environmental transport via surface water.

TH146

Following the fate of microplastics in a blue mussel exposure system

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Many laboratory studies have exposed marine invertebrates to microplastics and have reported ingestion as well as effects. A special focus has thereby been on the blue mussel *Mytilus edulis* and it is well known that blue mussels ingest microplastics of different sizes and shapes. Also, adverse effects on the animals' physiology have been reported. Particle concentrations in exposure tests are, however, often substantially higher than what is found in the marine environment. Furthermore, the fate of the tested particles in the exposure system is rarely investigated thoroughly. Both aspects are, however, important for evaluating observed effects. The aim of this study was therefore to quantify ingestion and egestion of microplastics in *M. edulis* at environmentally realistic particle concentrations and trace the fate of all particles in a controlled exposure system. Blue mussels were exposed to 50 μm polystyrene (PS) beads at concentrations of 5 or 100 beads L^{-1} for 10, 20 or 40 min and subsequently moved to clean seawater for 2h of depuration. After the respective exposure and depuration times, mussels were sampled to quantify the beads per animal. Additionally, the water bodies were filtered to retrieve all remaining beads. The number of beads in each compartment was quantified by counting them under a stereomicroscope. For this, the mussel tissues were enzymatically digested using a novel protocol with the protease Alcalase. This protocol proved to be highly efficient in digesting the tissue, while at the same time being easy to handle. It is thus promising to be used as a standard method. The quantification of PS beads in all compartments of the exposure system (i.e., mussels after exposure, exposure water, mussels after depuration, depuration water) resulted in a very high recovery and enabled the establishment of a mass balance of beads. This was achieved even at a low, environmentally realistic concentration. The distribution of the beads in the system was similar for both concentrations, indicating that mussels did not adapt their filtering activity. Two hours of depuration were not sufficient for egestion of the beads and should be prolonged. It is recommended that analyses of particle fate are incorporated more frequently in exposure studies as this can enhance our understanding of particle-organism interactions and observed responses.

TH147

Interactions and impacts of wheat plants grown in the presence of nanoplastic

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Plastic residues are highly resistant to degradation and persistent in the environment. Agricultural soils are an important sink for small particulate plastic (nano- and microplastic particles and microplastic fibers), either through the application of sewage sludge as amendment for nutrient replenishment or the wear and fragmentation of farming materials, such as mulch foils. A recent study has suggested that soil biophysical characteristics may be affected by the presence of particulate plastic (e.g. water holding capacity and soil structure), but it is not clear how crops are affected. In particular, in this study we aim to study the uptake and possible phytoxicity of nanoplastics in wheat plants. Because limited analytical techniques exist to measure nanoplastic particles, unique metal doped nanoplastic have been developed in our previous work, where the metal can be measured as a proxy for plastic in the system. A three-week hydroponic culture experiment was conducted where wheat plants were exposed to Pd-doped nanoplastics, added to fourth strength Hoagland nutrient solution at three different concentrations (0 mg. L^{-1}, 3 mg. L^{-1}, 30 mg. L^{-1}), and with two surface morphologies (either smooth or irregular). The zeta potential and agglomeration state of the nanoplastics and the pH of the nutrient solution were monitored during the culture. After harvest the following phytotoxicity parameters were determined: biomass, lipid peroxidation (TBAR´s) protein and chlorophyll content. Biochemical changes in plant tissues were studied by Fouried Transform Infrared Spectroscopy using Attenuated Total Reflectance (FTIR-ATR). Pd content in the roots and shoots were measured by ICP-MS, to quantitatively assess nanoplastic uptake. Nanoplastic distribution in the roots was studied by synchrotron micro X-ray fluorescence (μXRF) and scanning electron microscopy (SEM). X-ray micro computed tomography (μCT) was used to assess anatomical changes in exposed roots. In the nutrient solutions, the agglomeration state and pH increased during the culture. The increased agglomeration was consistent with the evolution of the zeta potential, which became less negative. Preliminary analysis on the harvested wheat plants showed no or limited phytotoxicity. μXRF showed nanoplastics sorbed on the root surface, which could hypothetically affect nutrient uptake. μCT and SEM-EDX data are currently being processed. This experiment is the first investigation on the possible uptake and effects of nanoplastics on plants.

TH148

Do microplastic fibers affect the toxicity of Ag nanoparticles to the water flea Daphnia magna?

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Microplastics (MP) are defined as plastic fragment waste having size < 5 mm. They have drawn scientific attention, as they can be abundantly found in the environment, emitted from different sources. One important source of MP is the release of fibers from textiles during wash. These fibers can reach freshwater ecosystems through the wastewater effluents. Moreover, the MP fibers can be emitted together with Ag nanoparticles (Ag-NP), since Ag-NP are incorporated in textiles due to their antimicrobial effects and are also released during the washing process. Therefore, this study aims at evaluating the joint effects of MP fibers and Ag-NP on the water flea *Daphnia magna*. Neonates (< 24h) were exposed to Ag-NP (2.56 – 250 μg Ag/L) and polyethylene terephthalate (PET) fibers (10 and 100 mg/L) in a full-factorial design, including controls. After 48h, the number of dead animals was recorded. The LC50 (95% CI) for Ag-NP in the absence of MP fibers was 204 (143-291) μg Ag/L, while values of 137 (111-169) and 164 (150-179) μg Ag/L were found in the presence of 10 and 100 mg/L of MP, respectively. Even though the LC50 decreased in the presence of MP fibers, no differences could be found among treatments, based on the overlap of the 95% CI. The main conclusions obtained in this study were: (1) MP fibers did not cause mortality of *D. magna* up to 100 mg/L when exposed alone; (2) PVP-coated Ag-NP (particle size of < 100 nm) used here was not as toxic as other Ag-NP reported in the literature; (3) the influence of MP fibers on Ag toxicity was not dose-related to MP fiber concentration in the water, as 10 and 100 mg/L of MP fiber produced very similar results regarding Ag toxicity. These are, however, preliminary results and the tests will be repeated to confirm these findings. Also, tests will be performed including ionic Ag (as $AgNO_3$) for comparison. Even though the number of studies is increasing, the toxicity of contaminants combined with MP is a very complex subject. Only a small number of toxicity studies have been conducted using irregular shaped MP, while most studies have used readily purchased spherical MP. Therefore, the outcomes from this study might give some insight into realistic exposure scenarios for daphnids. Due to the expected increasing levels of MP fibers and their high persistence in the environment, in case MP fibers really increase the toxicity of Ag-NP, their combined exposure can pose a risk to the aquatic organisms.

TH149

Biological impacts of bio-based and compostable microplastics on blue mussels (Mytilus edulis)

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The replacement of petrochemical plastics, owing to the severity of threat they impose to marine environment, to bio-based plastics need an evaluation for the toxic impacts of these new materials on marine wildlife. This study aimed to assess the toxicological effects of compostable plastic microparticles (MPs) on a marine shellfish, the blue mussel *Mytilus edulis,* combining biochemical marker analyses and a more global metabolomic approach. MPs of sizes ranging from 10 to 100 μm were produced from the fragmentation of polylactic acid (PLA). Each 15 individuals were exposed to no MPs, or MPs at two concentrations (10 μg/L and 100 μg/L) for 8 days. Significant decrease of AChE was observed in 10 μg/L compared to controls and 100 μg/L. These neurotoxic effects were similar as those depicted for petrochemical MPs. LMS and AcP activity were investigated to monitor the health of lysosomes of hemocytes. Insignificant variations of enzyme activity in mussels of all groups and no significant differences in lysosomal membrane structures were observed. Regarding oxidative stress, CAT, GST and SOD activities were significantly increased in 10 μg/L concentrations. These upregulations implied physiological challenges the organism faced after MP exposure. The OPLS-DA on metabolomic data allowed to discriminate the individuals according to their exposure condition. The loading plot highlighted 42 features down regulated in the individuals exposed during 8 days to MPs. Most of these molecules were identified as glycerophospholipids based on HRMS data. Glycerophospholipids are one of the major components of lipid membranes where they ensure several roles including regulation of the binding of protein to membranes, acting as messengers and participation in signaling such as cell cycle and appoptosis. To conclude, PLA microplastics showed effects on the blue mussel after 8 days of exposure at environmental doses with the detection of oxidative stress and neurotoxicity. Greater effects were shown at the lowest concentration (10 µg/L) compared to the highest concentration (100 µg/L). This observation could be explained by low filtration activity of gills at highest concentrations, as it was previously demonstrated for plastic nanoparticles. The lipidome of mussels was also impacted by MPs at both doses. Biochemical and metabolomic approaches appeared to be complementary tools in the assessment of environmental risk of bio-based microplastics.

TH150

Biological Effects of virgin and weathered Microplastic Particles and their Leachates in the green algae Scenedesmus vacuolatus

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Studies on the potential effects of microplastic (MP) particles in the aquatic environment are numerous. However, many laboratory studies apply pristine particles, which may be of limited environmental relevance given that factors such as UV light irradiation, mechanical stress, salinity and biofilm growth can influence the weathering of plastic debris. The aim of this study was to assess the impacts that weathering has on the effects of MP particles and their leachates on the unicellular green algae *Scenedesmus vacuolatus*. The presented work reports on two experiments performed on pristine and artificially aged plastic material: in the first experiment, focusing on the development of different size fractions during weathering LDPE and PET particles were exposed to UV (equivalent to 112 d outdoors). In a second, experiment leachates were obtained after the weathering of PE, PS, PP and PET as well as positive controls (equivalent to 510 days outdoors): (1.) Impact of MP particles on green algae: exposure to different size fractions of MP. Four different effect parameters (related to growth as well as photosynthetic capacity) for the different fractions were observed. The biological experiments were accompanied by an extensive characterization of the amount and size of polymer particles in each of the fraction. (2.) Mixture effects of chemicals liberated from the polymers during weathering toward algae. Chemicals built during UV-exposure and liberated in leachate water during simulated weathering of MP were enriched by solid-phase extraction. The concentrated leachates were then dosed into the algae assay. The results show that for LDPE and PET polymers the aging regime leads to shifts in particle masses contained in the different fraction, with a stronger impact of weathering on the PET. None of the fractions, irrespective of polymer type, impacted growth and photosynthesis of green algae. For the leachates, however, distinct effects were observed. The positive control used in the leaching experiments (electronic waste, milled keyboard) provided a proof-of-principle. Leachates from the mostly additive free reference polymers PE, PET, PS and PP showed higher effects in the UV treatments compared to the corresponding dark controls toward *Scenedesmus vacuolatus*. Our findings indicate that the biological effects of chemicals leaching out of weathering plastic material may have a stronger impact on algae compared to the weathered polymer particles.

TH151

Comparative toxicity of plastic additives toward Daphnia magna

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Worldwide, the amount of plastic production getting increased from 1.5 million at 1950 to 320 million tons at 2015. Plastics are used in many ways, such as packaging, building and construction, transportation, and so on. Recently, researchers have reported the significance of microplastics ingestion by aquatic organisms (e.g. fish, water flea, mussel). For instance, it was demonstrated that *Daphnia magna* can take microplastic fiber with size from 62 to 1400 μm. Additionally, acute toxicity of additives from polystyrene (PS) to *Ceriodaphnia dubia* was reported and LC50 of ethylbenzene leaching from PS was appeared to be 14 ppm. Most research focused on the adverse effect of micro- or nano-size plastics on aquatic organisms, while a few study reported the effect of plastic additives such as antioxidants, light stabilizer and thermal stabilizer. Especially, the study on the toxic effect of plastic additives on *D. magna* is very limited. *D. magna* has high sensitivity to pollutants and thus wildly used for toxicity testing. Therefore, the aim of this study was to compare the toxic effect of additives from plastics to *D. magna*. Acute and chronic toxicity testing was conducted according to the OECD guidelines to quantify the adverse effect of additives from plastics onto *D. magna*.

TH152

Microplastics as Pollutant Vector in Rivers and Oceans: Influence of Non-Linear Sorption and Coupled Mass Transfer

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Microplastic particles are ubiquitously detected in the environment. Despite intensive discussions, an assessment of their potential to transport contaminants in rivers and oceans is still needed. Thus, we aimed to combine measurements and modelling approaches to improve the understanding of pollutant sorption to microplastics. We measured sorption isotherms and kinetics in batch experiments with phenanthrene as typical hydrophobic contaminant and different types of particles, varying in size and material. While we observed linear sorption isotherms for polyethylene (PE), polyamide (PA) and polystyrene (PS) showed nonlinear sorption which we described best by the Freundlich and Polanyi-Dubinin-Manes isotherms, respectively. In order to evaluate sorption kinetics, we developed a model that describes sorption as a combination of two diffusive resistances, namely external mass transfer governed by diffusion through an aqueous boundary layer and intraparticle diffusion within the plastic. Which of these resistances controls the kinetics depends on the sorption strength, particle size, diffusion coefficients, and time. We applied semi-analytical and numerical methods to simulate coupled mass-transfer for both linear and non-linear sorption. Mass transfer of phenanthrene to PE shifts from film to intraparticle diffusion over time. By contrast, kinetics of PS and PA are limited by intraparticle diffusion over almost the whole time as diffusion coefficients are several orders of magnitude lower. The numerical code was successfully applied to PA and PS, allowing to describe the measured kinetics and obtain reasonable values for mass transfer and intraparticle diffusion coefficients. Subsequently, we used these coefficients to estimate the transport potential and time scales for microplastic-bound contaminants under environmental conditions in river and ocean settings. Whether the particles act as a passive sampler or transport vector is strongly dependent on particle diameters, partition coefficients, and intraparticle diffusion coefficients. The potential for long-range transport increases with the particle size squared and decreases with the intraparticle diffusion coefficient. On the other hand, smaller particles equilibrate fast and thus presumably reflect the ambient concentration in water, i.e. act as a passive sampler. Furthermore, the time scales imply that particularly larger particles have the potential to transport sorbed substances to remote areas.

TH153

Do microplastics contribute to organism exposure to contaminants? Modeling contribution of microplastic to environmental exposure & implications for prioritization within a risk-assessment framework

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Micro- and nano-plastic particles are ubiquitous in freshwater and marine environments, including soil and sediment compartments. There has been significant work, to date, to address the potential for co-contaminants in these systems (e.g., persistent organics (PCBs, PBDEs, PFAS), hydrocarbons (PAHs), pharmaceuticals, and heavy metals) to sorb onto these small plastic particles (SPP) and to be transported both within the environment and within organisms. **This body of work, however, has largely failed to answer the practical question – are these "secondary effects" of micro-plastic co-contaminants a significant factor in assessing the risk of microplastics to (human health and) the environment?** The purpose of this work is two-fold: First – to summarize available sorption and de-sorption data for various relevant classes of organic chemicals and metals onto both pristine and weathered plastic particles (PE, PP, PVC, PA) and compare these to the sorptive capacities of other relevant environmental solid phases (i.e., organic carbon, DOM, silica, etc…) in fresh and marine water systems as well as sediments. The second aim of this work is to place the sorption of these contaminants to SPPs into the larger context of total exposure to organisms, incorporating relevant environmental exposure data for SPPs as well as other environmental particulates with a focus on relative contribution to the overall body burden within organisms. The overall goal of this work is to provide support and guidance for prioritizing these "secondary effects" of micro-plastics relative to other considerations (i.e., particle size, polymer-type, surface charge, weathering, agglomeration, etc…) within a risk-assessment framework under development for micro-plastic hazard and risk assessment.

TH154

Broad chemical industry initiative to develop a prototype environmental risk assessment framework for Microplastics (MP)and engage in relevant LRI research.

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1. Development of a prototype Environmental Risk Assessment Framework for MP There continues to be increasing concern regarding the presence of MP in the environment. There are also still many knowledge gaps around the impacts of MP in the environment. To best address data gaps requires co-operation among all stakeholders engaged in advancing our mechanistic understanding of the environmental effects, fate, and exposure of MP. To most effectively set and use research priorities and capabilities, industry started to facilitate multi-stakeholder events and developed an organisational structure to quickly convert research priorities into projects. **2. Cefic-LRI research project ECO48: "Develop Fate and Transport Model for Microplastics in the Aquatic Environment" (250.000Eur/2y, start Q1-2 2019)** A regional and global scale environmental fate and transport model for MP is needed 1.Takes into account and leverages existing fate and transport modelling frameworks for similar particulate matter; 2.Identifies physical/chemical properties of MP that are useful for informing environmental fate and transport 3.Determines environmental characteristics that are useful for informing environmental fate and transport 4.Informs the expected environmental concentrations of MP in different compartments. ECO48 RfP 3. **Cefic-LRI research project ECO49: "Evaluate factors that determine the environmental hazards of microplastics" (400.000Eur/3y, start Q1-2 2019)** Phase 1: It is proposed that a comprehensive literature review be conducted in order to identify both key ecological hazard research gaps and the appropriate methodologies for conducting hazard tests with MP materials to fill these research gaps. Phase 2: Based on this literature review, targeted ecological hazard research should be proposed and conducted to evaluate how both intrinsic and extrinsic factors influence the effects of MP on sensitive environmental species. ECO49 RfP **4. Review the state-of-the-science for aquatic toxicity testing of particles and poorly soluble liquids** The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) initiated a Task Force (TF) to review the literature in relation to the aquatic toxicity testing of particulates, such as nanomaterials and microplastics, and poorly soluble liquids. The TF identified an urgent need to strengthen the quantitative and mechanistic understanding of the relationship between intrinsic physicochemical properties and observed adverse effects.

TH155

Microplastics in the Environment - A Literature Trend Analysis

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Microplastic pollution is a growing environmental and public health concern given the ubiquity of particles identified in environmental samples. While occurrence of marine microplastics has been documented in a number of regions, potential environmental and human health impacts are less well understood. The goal of this review is to characterize the current state of the peer-reviewed literature on microplastics and to identify key literature references as well as data gaps. An emphasis is placed on analytical techniques for environmental sampling, exposure considerations, environmental fate and transport, ecotoxicology, and potential human health impacts, key focus areas required to address a risk assessment framework for microplastics. >2500 articles were initially identified through Web of Science and Pubmed database searches. Following removal of 868 redundancies and exclusion of subject-irrelevant publications by manual curation, 1219 articles relevant to the search topic were retained. Software-assisted trend analysis in "Sciome Workbench for Interactive computer-Facilitated Text-mining" (SWIFT Review) revealed a proportionally strong increase in the number of new publications per year, a trend that is consistent with increased public awareness and media coverage on microplastic pollution. Intentionally manufactured and secondary, degradation-derived microplastic particles remain the primary focus in the literature (1102 hits). Concordant with the general increased interest in the topic, the secondary effects literature addressing additives, and vector potential for environmental contaminants and pathogenic bacteria, is proportionally increasing (163 hits). Subsequent categorization of articles in "Health Assessment Workspace Collaborative" (HAWC) indicated a strong focus in the exposure, environmental fate & transport, and ecotoxicology literature. Comparably fewer articles focused on analytical method development, and the most underdeveloped focus area was represented by the human health impacts literature. This review concludes with identifying relevant data gaps and research needs.

TH156

Health risk associated with plastic debris on the Island of Zanzibar

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Several studies have indicated that pathogenic bacteria bind particularly strongly to plastic pollution particles. This implies that plastic debris might serve as a vector for spreading of diseases with subsequent consequences for human health in polluted areas. In areas where (especially) poor people gather plastic waste to sustain a living for their family, and pathogens such as *Vibrio cholerae* are present, the plastic pollution paticles can be a vector for spreading disease among the local population. This potential human health risk of plastic pollution provides a substantial challenge, since there is a need to conduct mitigation efforts to preserve the environment and the ecosystem services it provides, and at the same time avoid enhanced risk of Cholera or other pathogenic bacteria such as pathogenic strains of *Escherichia coli.* Zanzibar is a pefect setting for this "one health" research project, as it features recurrent Cholera outbreaks as well as significant documented problems with plastic pollution and thus serve as a good area for a case study of this potential widespread problem. This project simultaneously aims to assess the risk of human health from plastic-associated pathogens to the local population in an epidemiological context and subsequently facilitate development of safe and effective mitigation efforts. This will be done by collecting plastic debris particles in relavant locations in Zanzibar and analysing which pathogens that are associated with the particles. The exposure to local communities will subsequently be assessed by interview and observation studies aiming at understanding how the local community is physically interacting with the debris under clean up operations. Upon having quantified the risk to the human population, we will seek to collaborate with the local community on educational campaigns about these risks, and to building the nessesary local infrastruture to handle the plastic pollution in a sustainable manner. This way the project aims at ensuring proper environmental protection and at the same time reducing health risk posed to the local community.

Bridging the Gap: Maximizing the Role of Mechanistic Approaches (Including Omics) for Better Chemical Safety Decisions Across Humans and Ecosystems (P)

TH157

Cytotoxicity of deep eutectic solvents to human skin cells

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Deep eutectic solvents (DES) emerged in the last years as surrogates for conventional solvents. Their tailor-made character turns them very attractive, being the possibilities of designing new DES almost endless. This feature allows DES to be used in chemical, electrochemical and material applications, as well as in health-related areas in the pharmaceutical, nutraceutical, and cosmetic industries. However, although DES have been touted as "green" solvents, several works with model organisms and human cell lines proved that in some cases their potential toxicity should not be neglected, and in any case it should be characterized prior to extensive application. In the present study, we used the premise of DES applicability in the cosmetic and pharmaceutical sectors. Two cell lines were chosen to work as a skin models (keratinocytes HaCaT and tumor melanocytes MNT-1), to assess DES cytotoxicity. The effect of three different hydrogen bond acceptors (HBA) ([Chol]Cl, [N_{1111}]Cl and [N_{4444}]Cl) and three different hydrogen bond donors (HBD) (hexanoic and butanoic acid, ethylene glycol, 1-propanol and urea) were evaluated through the common viability assay (MTT assay). Thus, the cytotoxicity of fifteen DES, as well as their starting materials, was assessed for both cell lines. Results were promising since [Chol]Cl and [N_{1111}]Cl- based DES showed good biocompatibility for the tested cells. [N_{4444}]Cl-based DES, however, showed cytotoxicity for both cell lines, with the HBA being the driver of the toxicity. Interestingly, some compounds increased cell viability in the HaCaT cell line. [Chol]Cl, ethylene glycol, hexanoic acid, urea, and all [Chol]Cl and [N_{1111}]Cl-based DES are such an example and should be considered as targets for future studies regarding their potential in skin regeneration. These results highlight the versatile character of some DES and their possible use in cosmetic or pharmaceutical formulations. CICECO and CESAM were financed by national funds through the FCT/MEC and when co-financed by FEDER under the PT2020 Partnership Agreement (UID/CTM/50011/2013 and UID/AMB/50017/2013). FCT funded IPE Macário, C Menezes, H Oliveira, AMM Gonçalves, JL Pereira and SPM Ventura (SFRH/BD/123850/2016, BI/CESAM/012/AMB/50017, SFRH/BPD/111736/2015, SFRH/BPD/97210/2013, SFRH/BPD/101971/2014 and IF/00402/2015), and supported the research through the project Ref. PTDC/ATP-EAM/5331/2014.

TH158

Molecular mechanism of Cadmium-TiO2 nanoparticle mixtures when co-exposed to the nematode Caenorhabditis elegans

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Nanoscale titanium dioxide ($nTiO_2$) is probably among the most relevant engineered nanomaterials with a projected accumulation rate in European river sediments of 2 $mg*kg^{-1}*yr^{-1}$ (1). To evaluate the environmental risk of $nTiO_2$, the impact of co-exposure with other aquatic contaminants like cadmium (Cd) should be considered. While Angelstorf (2) had shown that the toxicity of $nTiO_2$ (P25) to the nematode *Caenorhabditis elegans* increases under simulated solar radiation (SSR), Samet (3) found synergistic inhibitory effects on the nematode, when co-exposed to $nTiO_2$ and Cd. The effect again was only observed when worms were irradiated during exposure (inhibition of reproduction by 80 % after SSR, 20 % under solely dark conditions). We present here experimental data which may explain this synergistic effect: (a) Cd is known to compete with Ca for ion-channels due to the chemical resemblance of the two ions (4). It also induces intracellular Ca-signalling as part of protective cell processes (5). Thus, the effect of Cd/$nTiO_2$-mixtures on Ca-ion channels in the intestinal membrane of *C. elegans* was investigated using a known human ion channel (TRPM7)-blocker: NS8593. The combination of nTiO2 and NS8593 showed the same effects under SSR as the $nTiO_2$/Cd mixture, which supports our hypothesis that the mode of action involves this Ca-channel. To validate this result, other possible receptors and their antagonists were tested: Lanthanum for TRPM3-channels in the cellular membrane and Heparin for IP_3-receptors in the membrane of the endoplasmatic reticulum. (b) In order to understand, whether the radiation has any impacts on the chemical structure of $nTiO_2$-Cd-agglomerates, not-irradiated and irradiated samples were characterized by Scanning Electron Microscopy, Dynamic Light Scattering and Energy Dispersive X-ray Spectroscopy. To also test the influence of pH changes during gut passage of agglomerates, samples were tested at pH 4 and 7. (c) Further experiments are currently undertaken, looking into the impact of $nTiO_2$-Cd-agglomerates on membrane integrity applying propidium iodide to check for non-specific effects of the Cd/$nTiO_2$-mixture on the cells under SSR. (1) Sun, T. Y. et al. 2014. *Environ Pollut,* 185, 69-76. (2) Angelstorf, J. S et al. 2014. *Environ Toxicol Chem,* 33**,** 2288-2296. (3) Samet, K. Abstract SETAC Europe Annual Meeting 2017 Brussels. (4) Hinkle, P. M.et al. 1987. *J Biol Chem,* 262**,** 16333-16337. (5) Thévenod, F. 2009. *Toxicol Appl Pharmacol,* 238**,** 221-39.

TH159

A quantitative proteomics approach to study the toxicological pathways of silver nanoparticle: A comparison of human skin carcinoma cell to zebrafish embryo

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The advance in nanotechnology has improved the properties of conventional materials into a highly-reactive form, which broaden their applications into many fields. However, this development has also raised concern regarding their high-bioactivity could lead to the risk to both human and environment. Although many reports have addressed the adverse effects from nanoparticles (NPs), the underlying mechanisms of these observations were unclear. Moreover, the majority of nanotoxicological studies were conducted under the unrealistic condition, which only predicted the worst-case scenario of NPs exposure, therefore the relevant toxicological effects from NPs might not be comprehensively understood. In this study, the molecular effects of silver nanoparticles (AgNP) were investigated under the ecologically-relevant condition in both human skin cell and environmental model, i.e. zebrafish embryo, to compare the toxicological pathways by using quantitative proteomics approach. The comprehensive sets of differentially expressed proteins were identified from HDMS-LC-MS/MS, which were further categorised by their molecular function using Progenesis® QI for Proteomics. The toxicological pathways of AgNP in both human skin cell and zebrafish embryo were indicated by STITCH protein-ligand interaction algorithm. Moreover, the enrichment analysis based on KEGG pathways were performed, in which the difference in toxicological pathways could be reported. The predominant pathway analysed from human skin cell was DNA damage, whereas oxidative stress was the major toxicological pathway observed in zebrafish embryo. The similar protein pathway disrupted by AgNP in both models was the metabolic process. Although the difference in molecular pathways was established between human cell and zebrafish embryo, it confirmed the occurrence of adverse effects from AgNP, specifically in the low concentration. Moreover, metabolic process is important to maintain cellular homeostasis, as well as the development of zebrafish embryo. The future study on metabolomics would be constructive to this finding. These omics approaches would be powerful to screen safety of other NPs, which will be applicable to help design the effective and harmless NPs in the future.

TH160
Dreissenid mussels on sertraline medication: does the mode of exposure influence their biological responses?
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The presence of pharmaceuticals in aquatic ecosystems is now established. However, the risks to aquatic organisms after chronic exposures to low environmental concentrations are still unknown and therefore remain to be determined. Pharmaceutical releases into the environment are not always continuous and can also be by pulses. In this context, both their concentrations, the duration of exposure and the frequency of this exposure may influence the biological responses of non-target organisms. However, up to now, laboratory experiments mostly mimicked continuous exposure. Therefore, it is important to determine if the mode of exposure to pharmaceuticals may affect the biological responses of non-target aquatic organisms. In order to provide answers to this question, two freshwater bivalves of the genus *Dreissena*, commonly used in ecotoxicological studies (the zebra mussel, *Dreissena polymorpha* and the quagga mussel *Dreissena rostriformis bugensis*) were chosen as model organisms. These two species have different life history traits and thus, may respond differently to the exposure. The bivalves were exposed to sertraline during 28 days, either continuously or by pulse every 5 days. Sertraline (Zoloft), a selective serotonin reuptake inhibitor, is an antidepressant commonly prescribed worldwide to treat mental disorders. During this experiment, several biological responses were followed every week, such as the contents of energy reserves, the activities of antioxidant defenses and lysosomes, or the behavior of bivalves. The concentrations accumulated by the bivalves were also measured. The first results show a higher sensitivity of *D. r. bugensis* to sertraline exposure than *D. polymorpha* during the spawning period.

TH161
Gene transcription analysis of Daphnia magna exposed to carbendazim: a multigenerational approach
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Despite all the progress in agriculture, pesticides are still used and consequently continuously released into the environment, with possible long-term consequences for aquatic organisms. One of the pesticides still applied in several crops in some countries is the fungicide carbendazim, ending up in surface waters with concentrations reaching 5 μg/L. In the present study, *Daphnia magna* (clone k6) was exposed to a sublethal concentration of carbendazim (5 μg/L) for 12 generations. Gene expression alterations induced by this compound were assessed in the F0 and F12 generations using a *D. magna* custom microarray. Results revealed that carbendazim caused changes at the gene expression level in both generations. Genes involved in response to stress, DNA replication/repair, neurotransmission, protein biosynthesis, ATP production, lipids and carbohydrates metabolism were the most affected in both F0 and F12 generations. Compared with the F0, a lower number of differentially expressed genes was observed in the F12 generation exposed to carbendazim. The exposure of daphnids to carbendazim did not cause a stable change in gene expression from F0 to F12 generations. Effects at the gene expression level were already detected in the F0 generation after a short-time exposure (10 days), highlighting the advantages of using high throughput tools for early warning analysis and adding value to risk assessment procedures. These gene expression data complement the available information on effects at different higher biological levels oforganization and will therefore improve the understanding of effects of carbendazim under a multigenerational exposure scenario.

TH162
Chemical prioritization by computational transcriptomics: a two case studies
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Thousands of chemicals used worldwide have the potential to harm human health and ecosystem functioning. Toxicity information for most of these compounds are currently lacking. However, due to experimental costs and time and in agreement with the requirement of the 3Rs framework, it is not practical to evaluate toxicity for all the compounds to which humans and ecosystems are potentially exposed. Developing alternative approaches with the ability to prioritize harmful compounds is of paramount importance. In a first study, a *de novo* transcriptome assembly was performed to investigate signals involved in ovary maturation in the swordfish (*Xiphias gladius*). By focusing on differentially expressed genes, a pathway analysis was employed to investigate the molecular mechanisms driving the transition from immature to mature ovary. Genes found to be differentially expressed hence playing a key role towards maturation where used to interrogating the Comparative Toxicogenomic Database (CTD) in order to identify compounds with the potential to affect ovary maturation. Chloropicrin and Cyclosporine were identified as the best candidate compounds with the ability to disrupt ovarian maturation. In a second study, we developed a dynamic model of ovary development linking gene transcription to key physiological endpoints, such as gonadosomatic index (GSI), plasma levels of estradiol (E2) and vitellogenin (VTG), in largemouth bass (LMB) (*Micropterus salmoides*). By mapping the responses of a transcriptome from LMB collected from a polluted site, we identified a sub-network that closely linked gene expression and physiological endpoints and by interrogating the CTD, Quercetin and Tretinoin (ATRA) were identified as two potential candidates to elicit reproductive disruption. Predictions were validated by qPCR in ovary and in the liver of both male and female LMB exposed to Quercetin and Tretinoin. Both compounds were found to significantly alter the expression of key endocrine genes. These studies demonstrate that by utilizing computational approaches and online knowledge bases to understand the underlying molecular response of organisms, it is possible to identify putative chemical candidates that may impact reproductive health. This approach is highly relevant for classifying chemicals prior to conducting risk assessments, and we propose that this is a viable approach for chemical prioritization, reducing animal numbers, and developing safer chemicals in the public domain

TH163
Adverse outcome pathways for non-chemical stressors: the case of radiation
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An adverse outcome pathway (AOP) mechanistically organizes the existing (eco)toxicological information from different levels of biological organization, similarly-acting stressors and phylogenetically conserved taxa into a linear pathway by connecting a molecular initiating event (MIE), a series of subsequent key events (KEs) and an adverse outcome (AO) of regulatory concern. The AOP framework has promoted mechanistic understanding of toxicity, and the development of *in vitro* and *in silico* approaches for more efficient hazard and risk assessment of chemicals. However, organisms in the environment are not only exposed to chemicals, but also a multitude of stressors such as radiation, abiotic and biotic factors. These non-chemical stressors may cause adverse effects and affect the toxicity of chemicals. Since the current AOP framework is chemical-centric, there is need to expand it to non-chemical stressors for future application of AOPs in hazard and risk assessment of multiple stressors. The present work was therefore conducted to develop AOPs for non-chemical stressors using radiation as a starting case. Both ionizing (e.g. gamma) and non-ionizing (e.g. UVB) radiation are known to induce reactive oxygen species (ROS) and DNA

damage as common MIEs and eventually lead to reduced survival, growth and reproduction as common AOs. Extensive literature survey and data mining were performed to summarize existing knowledge and to develop a conceptual AOP network for ionizing and non-ionizing radiation. Experimental evaluation of the AOPs were conducted using the aquatic crustacean Daphnia magna as the forecaster species, and gamma and UVB radiation as the prototypical stressors. A suite of effect assessment tools such as the ROS formation assay, DNA damage assays, apoptosis assay, lipid peroxidation assay, mitochondrial membrane potential assay, ATP assay, lipid storage assay and biomarker gene expression assay were used to populate KEs of the conceptual AOPs with experimental data. The overall weight of evidence was assessed using the Bradford Hill Considerations and applicability domains defined using the NIVA Risk Assessment Database (NIVA RAdb^{TM}), which collects and organizes (eco)toxicological data from both in-house studies and public databases. The AOPs have been submitted to the AOPWiki for public review. This study has for the first time developed AOPs for non-chemical stressors, and provided mechanistic knowledge of radiation-mediated adverse effects.

TH164

Dose-response Curves for -OMICS data in soil quality assessment (DrComics)

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Traditionally, phenotypic endpoints of test organisms, such as survival, reproduction, and growth, have been used for the assessment of a compound's toxicity. However, these endpoints are influenced heavily by environmental factors such as seasonality, climate and soil type. Moreover, human activity releases more pollutants, and varying mixtures thereof, into the soil than can be tested under lab conditions. Our research consortium seeks to develop robust metrics for toxicity testing by trying to find dose-dependent effects on the gene regulation of springtails (*Folsomia candida*) upon insecticide exposure, i.e. neonicotinoids. We are measuring reproduction and obtain transcriptomics and proteomics data from springtails under a broad range of stress intensities caused by neonicotinoids as single compounds and in mixtures with other neonicotinoids or with known metabolomic inhibitors. These data will be used to help scale current dose-dependent methodology to the omics era. This allows us to identify patterns of gene regulation which remain robust under shifting metabolomic conditions and at various stress intensities. The results of this work can lead to the development of methods for rapid and cheap soil quality assessment such as insecticide detection. Moreover, it may provide policy makers with metrics of soil pollution that are biologically relevant, i.e. stress related gene regulation patterns, instead of dose limits derived from testing under controlled conditions of single compounds in artificial soils.

TH165

The Eco'n'OMICs project - an OMICs-based screening approach for ecotoxicological risk prediction

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Active substances of pesticides, biocides or pharmaceuticals can display adverse effects in non-target species, which may threaten populations with far-reaching consequences for the ecosystem. Therefore, european legislation requests manufacturers to provide data for environmental risk assessment of active substances in order to become registered. The corresponding OECD tests are time- and cost-consuming and come along with a substancial number of animal tests. Thus, they are performed only in the end of industrial substance development, bearing the risk of a failing registration due to proven adverse environmental effects. The Eco'n'OMICs project aims at an early ecotoxicological risk prediction for active substance precursors based on substance-induced molecular changes in aquatic model organisms. We combine recent transcriptomics (RNA-Seq) and proteomics (LC-MS/MS) technologies with ecotoxicological approaches to identify gene expression changes induced by a number of ecotoxicologically well characterized reference substances. These substances are selected to be representative for substance classes and to cover a large range of different modes-of-action. The identified substance-specific molecular signatures are linked to a data base with adverse environmental effects and the compound structure. These biomarkers will be used to develop a targeted molecular screening approach for ecotoxicological risk prediction. Together with a structure-based prioritization of substance precursors, the availability ofsuch a molecular screening will enable for a time- and cost-efficient, development of environmentally friendly active substances.

TH166

Effects and concentrations of the ß2-adrenergic agonists salbutamol in fish - do they confirm the fish plasma model?

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The hypotheses of the fish plasma model (FPM) were tested using the anti-asthmatic pharmaceutical salbutamol, an agonist of the ß2-adrenergic receptor. Salbutamol was tested in fathead minnow *(Pimephales promelas)* ina flow-through system at concentrations from 0.0066 to 4.1 mg/L over 120 days. Survival was recorded daily, and the apical endpoints biomass and length were evaluated at test end. From all fish, blood was sampled at test end and subjected to chemical analysis of salbutamol. In addition, triglycerides were determined in whole body of the fish after sampling of blood. The measured fish blood plasma concentrations in relation to the measured water concentrations were in reasonable agreement with the predictions of the FPM. This confirms the hypothesis of the FPM that fish plasma concentrations can be predicted from water concentrations using a descriptor for lipophilicity. No significant effects were detected on apical endpoints in fish (i.e. biomass and length) after 120 days of exposure up to a concentration of 4.1 mg/L. Determined plasma concentrations in fish, however, reached levels that are approximate to those in human patients under salbutamol treatment. Hence, the hypothesis of the FPM that no adverse toxic effects occur below human therapeutic concentrations was consistent with the results of this experiment. Determination of total triglyceride levels in whole body indicated significant metabolic impacts of salbutamol at the highest tested concentration of 4.1 mg/L. This finding provides evidence that sub-lethal effects in fish are linked to the pharmacological target as salbutamol is also known to induce cardiovascular and metabolic side effects in humans. Overall, the present study provides supporting evidence for the FPM applied to a therapeutic class of pharmaceuticals for which so far little information was available. With regard to environmental concerns, it can be stated that concentrations at which effects were observed after long-term exposure of early life stages of fish were significantly above the predicted and measured environmental concentrations.

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TH167

Revisiting the Mycophenolic Acid Chronic Surface Water PNEC in the Light of Immunotoxicity

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A surface water predicted-no-effect–concentration (PNEC) for mycophenolic acid (MPA) was derived from chronic no-observed-effect-concentration (NOEC) data developed in ecotoxicity tests with green algae, cyanobacteria, daphnids and fish that were performed in accordance with OECD test guidelines under GLP quality assurance. MPA is used to prevent organ rejection in solid organ transplants as its mode of action is immune inhibition. During planning the chronic tests, an additional investigation of immune-toxicological endpoints was envisaged. This comprised an assessment (up- or downregulation) of immune-related and other gene transcripts in the young fish at the end of the early life stage test as well as a parallel daphnid reproduction study with daphnids being challenged with the parasitic bacterium *Pasteuria ramosa* in addition to MPA exposure. The immune-related endpoints are compared with the standard apical endpoints from the chronic tests, in order to address the question whether standard ecotoxicity endpoints and the PNEC based thereon are also protective for immune-related effects.

TH168

Mechanisms of Action: a new classification scheme unifying human health and ecotoxicology, supporting non-animal testing approaches: example of the use with Fish Embryo Toxicity test.

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The Mechanisms of toxic Action (MechoAs) scheme has been developed mainly based on toxicological data on fish and mammals, and other species when available[1,2]. Therefore, in the majority of cases, it is capable of predicting both the differences and similarities in MechoA between these species and can be used in cross-species extrapolation. Some chemicals can be identified as sharing the same key events in fish and mammals, while others have very different AOPs, which potentially leads to differences in toxicity between species[1,2]. For example, an adaptation of the Fish Embryo Toxicity test (FET) can be used to study the teratogenicity, neurotoxicity, endocrine disruption and genotoxicity potentials of chemicals and the subsequent results could be extrapolated to humans[3]. Nevertheless, some applicability domain restrictions of this method have been stressed, notably a lack of predictivity for neurotoxicity[3]. Moreover, fish embryos have similar, if more limited, metabolic capacity than adult fish together with various organs which are not fully developed and at the pre-hatch stage with a thick protective membrane (chorion), all resulting in a difference in ADME profile. This adds another layer of complexity when trying to predict toxicity mechanisms in mammals from studies on fish[3]. Some case studies are given where the results from a FET test would not correctly predict the toxicity mechanisms of the substance of interest. Many of these cases can be identified through the use of the MechoA scheme, which can be used for free online[4]. Thus, the use of MechoAs is proposed, first to support and interpret FET test outcomes, then to ensure their extrapolation to another species is relevant depending on the expected AOP. Therefore, MechoAs can be used in a transversal language, supporting the FET test to help to unify both human and environmental toxicology. (1) Bauer *et al*. A New Classification Algorithm Based on Mechanisms of Action. *Comput. Toxicol.* **2018**, *5*, 8–15. https://doi.org/10.1016/j.comtox.2017.11.001. (2) Bauer *et al*. High-Accuracy Prediction of Mechanisms of Action Using Structural Alerts. *Comput. Toxicol.* **2018**, *7*, 36–45. https://doi.org/10.1016/j.comtox.2018.06.004. (3) Braunbeck *et al*. The Fish Embryo Test (FET): Origin, Applications, and Future. *Environ. Sci. Pollut. Res.* **2015**, *22* (21), 16247–16261. https://doi.org/10.1007/s11356-014-3814-7. (4) KREATiS. *iSafeRat® Online* v1.0; 2018. https://isaferat.kreatis.eu/

TH169

From gene orthology to functions and pathways: a new framework to identify conservation of toxicity pathways across species

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There is a growing recognition that an improved understanding of both molecular targets and pathways in the context of the Adverse Outcome Pathway (AOP) framework will allow for a more informed extrapolation to unravel impacts across chemicals and species. However, current approaches mainly focus on historical research that was not specifically intended for AOP development, leaving present methods deficient to adequately assess impacts across the whole chemical and relevant species space. An increased understanding of cross-species conservation of pathways would catalyse a tangible shift toward adopting Next-Generation Risk Assessment (NGRA) approaches. Genomes for a vast collection of different organisms are already publicly available, but there is still an overall lack of functional annotation. Additionally, although gene function can be inferred from sequence-homology, such approaches are based on several assumptions (not always correct) and have limited application. It is also important to consider that even though upstream signalling processes are likely to be similar across species, downstream processes may diverge, resulting in very different apical outcomes. Understanding the similarities / dissimilarities in physiological cascades of whole pathways across species is critical to assess conserved response patterns and associated outcomes. In this perspective, gene functional families may offer a more relevant metric of relative pathway conservation and potential perturbation, than the individual gene level. The overall goal of this project is to reduce the uncertainty inherent to cross-species extrapolation by utilising existing data to add evidence to the process of functional pathway re-construction. We propose an integrative use of available knowledge from different disciplines i.e. comparative biology, functional genomics and bioinformatics and chemistry, to create a new (multi-species) functionally-driven "orthology" framework to identify the conservation levels of pathways of toxicological concern. Data derived both experimentally and computationally, looking at genes/proteins, families and pathways and comparisons across species will all contribute to a weight of evidence approach to support risk-based decisions using conservation of pathways across species and improving the confidence in how we can use data from multiple sources.

TH170

RT-PCR arrays: a useful tool to analyze toxicity on invertebrates

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Most of the studies of environmental toxicology use ecologically relevant endpoints to study the effects. However, the molecular level is often neglected because of the lack of enough previous information to perform the experiment. For a long time, molecular analyses have been limited to biochemical methods detecting enzymatic activities but technological advances now allow to extend them to other molecular fields, like gene expression. By the end of the 20th century, massive sequencing methods were used to obtain the genome of different species. From there, next-generation sequencing methods (NGS) has evolved to provide transcriptomes of almost any organism thanks to new sequencers and bioinformatics. A final step in these advances has been mapping the reads of a biological sample back to the transcriptome in order to reveal the presence and quantity of RNA at a given moment, rendering thus an estimate of gene expression. NGS is becoming a methodology of the election in many studies, being now affordable for many laboratories. However, it is still too expensive when the number of samples to be analyzed is high; something usual for environmental toxicology. Multiple conditions need to be analyzed in order to obtain robust conclusions. Then, alternative methods are necessary to analyze gene expression with a balance between cost and a high number of genes that provide a good picture of cell metabolism. Invertebrates account for most of the species of animals and are key elements in ecosystems. Many transcriptome projects are available for several invertebrate species in databases ready to be used by researchers. Most transcriptomics studies focus on the identification of putative pathways or cell processes by massive analysis, often with homologies to proteins not confirmed. The sequences are rarely studied in depth. Here the transcriptome of *Chironomus riparius*, an insect, has been analyzed to identify genes and design arrays covering cell processes relevant to toxicant response. The arrays have been tested and the results obtained are presented. The workflow used in this work can be implemented in other species whose transcriptomes are available in databases. This way the value of the available sequencing projects can be increased, improving our knowledge about these species offering new tools for basic research and environmental toxicology studies. Funding: Ministerio de Economía y Competitividad, CICYT (Spain), CTM2015- 64913-R.

TH171

Quantifying Ecosystem Responses to Stress: An Environmental DNA (eDNA) Approach

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To better understand ecosystem responses to anthropogenic stress, new biomonitoring methods are needed that assess biodiversity in a quick and cost-efficient manner. The emerging field of environmental DNA (eDNA) potentially offers such an approach, as it may provide rapid, low-cost identification of species present in an area. To test the effectiveness of this new technology, eDNA was collected from ponds along an acid-mine drainage (AMD) gradient within The Wilds Conservation Center (Cumberland, OH). Here there are several AMD-gradient watersheds, with each system often consisting of one AMD-affected headwater pond that filters downstream to several smaller ponds where pH returns to background levels. We previously observed fish recolonization downstream from AMD sources and plan to use eDNA samples to quantify biodiversity in fish, amphibian, and invertebrate communities along a specific AMD gradient. The gradient here consists of 5 primary ponds separated by abandoned beaver dams each with highly different AMD characteristics. The goal of this proof-of-principle study is to estimate biodiversity and community structure at each pond using eDNA and then compare these results with traditional ecological assessment methods (e.g., netting, electrofishing, invertebrate sampling). Water quality parameters (pH, metals, conductivity) were also assessed along the gradient for comparison to derived community structures. The main source of metal contamination in our gradient is manganese (~6-10mg/L) with aluminum (~1-4mg/L) and iron (~0.5-3 mg/L) contributing as well. pH values range from 3.5-7.5 across the 5 ponds that were sampled. Our workinghypothesisis AMD reduces biodiversity and simplifies community structure at ponds closest to the AMD source and that these ecosystem changes can be detected via eDNA. Results from this study will provide a wealth of species information to be used in future remediation efforts by The Wilds. The extensive species data along a pollution gradient will allow for construction of site-specific species sensitivity distributions (SSDs) for pH, metals, and other AMD characteristics. Knowing which levels of AMD correspond to desired community structures can help create AMD benchmark levels for any future attempts to restore affected areas.

TH172

SETAC OMICS Interest Group

B. Campos, Unilever R&D / Safety and Environmental Assurance Centre SEAC

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Communicating Science, Failures, Fortune and Risks to Improve the Value of Environmental Research to Society (P)

TH173

How does knowledge about SVHCs affect individual consumption?

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Many consumers with minor knowledge in chemistry have difficulties in understanding information about hazardous substances in everyday products and in using this information properly. We conducted a survey among 1321 consumers who showed high motivation and interest in harmful substances in everyday products. We laid a special focus on the consumers' awareness of their right to inquire whether a consumer article contains substances of very high concern

('SVHC right to know') according to the European chemical legislation. There seems to be a big gap between the general desire to know more and the extra efforts needed for learning more: Every second participant declared that she or he wanted to know more about SVHC containing articles, whereas only four per cent of them indicated to use the SVHC right to know. We found that knowledge about SVHCs in articles would very much affect participants' behavior. Only one percent of all participants indicated that they would buy an SVHC-containing article. Ten per cent would even throw SVHC-containing articles, which they had bought already, into the garbage. We found significant differences in answers given by persons with very good self-reported knowledge in chemistry ("chemists") compared to those given by "non-chemists". Twice as many non-chemists compared to chemists would neither buy, nor use SVHC containing articles which they had bought already. Apparently, survey participants - even persons with good self-reported chemical knowledge - were not aware that today SVHCs are present in most electronic articles, vehicles and many textile articles. It seems to be very difficult for consumers to interpret the information about the SVHC content in articles and use this information for a suitable risk reduction behavior.

TH174

Lessons learned from bottom-up communication - Demands of information recipients must be taken seriously

U. Klaschka, University of Applied Sciences; S. Hartmann, University Ulm

We present a case study where a specific regulation concerning communication to consumers is scrutinized. We asked 1321 highly motivated consumers about their awareness of harmful substances in commodities. This online survey focused on the right to inquire whether a consumer article contains substances of very high concern ('SVHC right to know') according to the European chemical legislation. In general, the results of this survey showed that the 'SVHC right to know' is very complicated, also for motivated consumers, and we must assume that only a small minority of persons understand this provision correctly. Participants could indicate whether and how the 'SVHC right to know' should be improved. Only 1.6% of the study participants declared that the 'SVHC right to know' is good as it is. A third of the participants indicated that they did not use it and had, therefore, no suggestions for improvements. Nearly half of the respondents wished that substances of very high concern should be prohibited as soon as possible. On average, every participant gave two suggestions to improve the information on the packaging. More than a third of participants demanded more controls by authorities. Participants had a low interest in smartphone applications to inquire about SVHCs in articles (less than a fifth). There were significant differences in several answers given by persons with high self-reported knowledge in chemistry compared to answers given by non-chemists. Lessons learned: Procedures and legal provisions that are evident to experts are not necessarily so for laymen. Bottom up communication (asking recipients of information about their demands) is urgently needed for regulations that involve consumers. The differences in the answers given by chemists and non-chemists question whether decisions in environmental policy which are usually based on suggestions made by chemistry experts are suitable for stakeholders with little or no knowledge in chemistry. Considering the heterogeneity of the communication recipients and respecting their needs should be key elements in the implementation process of environmental policy to ensure that science-based solutions meet stakeholder needs.

TH176

How do we communicate chemical status of waters in Europe?

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Harmonized assessment of the quality of European waters has taken large steps in the 2010's with commonly defined Environmental quality standards (EQS) within both Water and Marine Directives (WFD, MSFD). However, monitoring practices, preferences and thresholds still vary between countries leading to different "chemical status". It is now evident that persistent, bioaccumulating and toxic compounds (ubiquitous PBTs) determine the non-compliance of chemical status of surface waters all over Europe – if measured. We have measured these contaminants (Hg, PBDE, PFOS/PFAS, HBCDD, PCDD/F) in perch trough out Finland during this decade. There are several examples of communication risk in assessing the chemical status: EQS set for human exposure, while food authorities have not set limits (PBDE, PFOS). EQS for PAHs is adopted from food sector, some PAHs are found in molluscs, but the indicator (BaP) is hardly found in our experience. In the 2nd WFD status assessment, mercury was estimated to exceed the EQS in ca. 50 percent of the waterbodies in Finland. Without calculated background concentration adopted in Finland, Hg would exceed the EQS all over country – as it would exceed anywhere in Europe, which is the case for PBDE as well. Also the strict dichotomy (only good/no-good) in status assessment is problematic to communicate, when data oscillates close to the EQS value. Conventions and directives thirst after proof of the long-term changes of restricted PBT substances. In the absence of multi-decade data on these substances in biota the historical development of contaminant fate can only be confirmed using undisturbed sediment stratigraphy. Risk management is not possible without first knowing where the problems are. Screening and monitoring will help us to focus the risk reduction and risk communication, but the principles should be similar geographically and among policy sectors (environment vs. food), especially if the target exposure end-point is the same – human health. Revision of the WFD will most likely include also a revision of the EQS derivation principles as well as the updating of individual EQS values. Increasing (eco)toxicological data can change the EQS values of the currently listed priority substances and also bring new substances in.

TH177

Managing chemicals in circular economy - communication actions are needed

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Circular economy (CE) development has significant environmental, economic and social benefits. The CE is paradigm for economic development, aiming to system change from linear to cyclical closed-loop systems. It is crucial to minimize the intake of natural resources. Opportunities in remaining the value of materials have driven the political decisions to set high percentage targets in recycling materials. However, reusing and recycling products and materials that might contain hazardous substances need risk management. Some of the most dangerous substances, such as persistent organic compounds (POPs) and substances of very high concern (SVHCs) remain as legacy in materials that have long life cycles. Environmental, health and occupational safety needs promotion in same time as promoting towards CE. The project 'Managing chemicals and ensuring safe and sustainable economy SIRKKU' seeks to promote CE by enhancing the management of chemicals. Main aim is to find ways to enhance chemical management and knowledge of hazardous substances especially in S/M companies. There is crucial need for new kind of communication. We investigated the present management practice regarding hazardous substances in companies by electronic questionnaire. Secondly, a workshop for authorities and experts was arranged. Discussions related environmental risks, health and especially occupational health, and various chemicals of concern in different fields of industries. As a preliminary result, we can conclude that the industries wish to have science based knowledge of wider impact assesments, LCA perspective and the potentials for standardising recycled materials. By the people operating in the field was especially required expert support e.g. pragmatic councelling. In the next steps the project is investigating 1) the Applicability of identification, separation and treatment processes (technical point of view), 2)Work safety risks associated with the use of recycled material, 3) Regulatory requirements and the development of policy instruments and 4) Impacts on environment and health. The investigations are in process and the results will be discussed with the construction and demolition industry in workshops. Transition to sustainable CE requires actions to support industries. Increase in awareness of hazardous substances in CE need communication actions. New technical development and occupational safety instructions in CE related work are essential for safe CE.

TH178

Framework to support creation of Safe and Sustainable material Loops in a Circular Economy

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Recycling options for waste streams are often assessed in terms of either their sustainability or their safety. These concepts are usually assessed by separate and specific methods, requiring specific expertise. The Safe and Sustainable Loops (SSL) framework is developed to provide one coherent and practical approach for policymakers, regulators and industry to assessing safe and sustainable reuse of materials recovered from waste streams. The framework consists of seven different modules. Some modules address the potential sustainability benefits related to reduced environmental impact and increased resource efficiency, while others address chemical or microbiological safety concerns. The chemical safety concerns can, for instance, be due to the presence of and potential exposure to substances of very high concern (SVHC). An example of an outcome of such an integral assessment is the clear benefit in terms of CO_2 footprint to recycle Polystyrene foam, while the assessment also shows that there is a clear challenge to deal with the presence of the flame retardant HBCDD (a SVHC used in the foam), posing a safety concern. The integral assessment shows that by extracting HBCDD with the solvolysis technique, this concern is adequately addressed, while the environmental benefit is maintained. Two other case studies are conducted: phosphate recovery from wastewater and rubber granulate from old tires. Some challenges for further rollout of the framework are the availability of assessment criteria and the need for guidance for practical use by different stakeholders. The developed framework aims to support evidence-based decision-making towards responsible and efficient use of materials and resources, and helps to address safety concerns by providing the context for further implementation of risk management options. This is a requirement for the transition towards a circular economy.

TH179

Environmental classification and labelling under the CLP Regulation: key principles, case-studies and downstream consequences

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Regulation (EC) No 1272/2008 (the CLP Regulation) refers to the classification, labelling and packaging of substances and mixtures and was introduced to ensure a high level of protection of human health and the environment, as well as the free movement of substances, mixtures and articles. Under CLP, hazard information on physical-chemical, environmental and human health hazards is compared to established classification criteria, described in Annex I of the CLP Regulation. Environmental classification refers to short- and long-term hazards to the aquatic compartment and "taxifies" aquatic hazard into different categories based on information on aquatic toxicity (acute and chronic), aquatic degradation and bioaccumulation. The key information relating to these endpoints will be presented and the key aspects taken into account during evaluation will be outlined, with a specific focus on the different related decision schemes. Different studies will be presented to highlight the application of the legal criteria for classification and de-classification (removal of existing classification). Different key considerations and encountered issues, both from a data-holder and from a regulatory perspective, will be reported and further analysed. Mixture classification principles will also be introduced and downstream implications elaborated on by use of example cases. Key hazard communication aspects will be discussed, mainly referring to labelling and its implications for the different actors. Importantly, downstream consequences of environmental classification and labelling will be tackled. These may refer to obligations/ implications to other chemical regulations for example, the Biocidal Products Regulation, the PIC Regulation, waste legislation, etc. Finally, the interlinks with the Seveso Directive will be highlighted in terms of containment, transport and general handling of substances with different environmental classifications. It is hoped that the interplay between (good quality) environmental information, hazard assessment and identification as well as hazard and risk communication will be highlighted.

TH180

Condensed, yet reliable - providing information of environmental safety of nanomaterials on a web-based knowledge base

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Nanomaterials have found their way in our everyday life, and are on the market in numerous applications, ranging from electronics to health care and environmental remediation techniques. Nanomaterials offer a great innovative potential and they are assumed to be beneficial to mankind and the environment by e.g. reducing material usage and energy consumption. As ultimately nanomaterials will enter the environment, concerns on transport, fate and adverse effects towards environmental organisms were raised. This initiated various research effort in the field, which to date resulted in an enormous body of scientific literature on this topic. Nonetheless, citizens in general, consumers of specific products, but also journalists or scientist from other fields often miss condensed, reliable and understandable information on nanomaterials and their potential effects on the environment. Hence, the DaNa2.0 project with support from many experts aims at providing such information for a broad audience on a web-based knowledge base (www.nanoobjects.info). One major challenge the DaNa 2.0 team is facing is the retrieval of relevant information from the continuously increasing collection of nanotoxicology related publications. In this context, a fast and effective information query for the evaluation of nanosafety related data is a first and very important step for activities such as the DaNa knowledge base. To facilitate the search and selection process, a novel IT-tool is currently under development, which will allow for an intelligent, concept-based search approach by means of extracting more semantic information from the text. This will enable the user to apply words describing the concepts and not just the search terms he is looking for complemented by an auto-completion system and further analysis tools for larger document sets. This poster intends to present an overview on the web platform that offers easy-to-understand, up-to-date and quality-approved information on 26 market-relevant nanomaterials concerning their effects on safety of humans and the environment. Specifically, the challenges in providing condensed but at the same time reliable information from a huge body of literature are being addressed. The knowledge base www.nanoobjects.info is making an important contribution to science communication in the field of nanosafety. *Acknowledgement:* The funding for the DaNa2.0 project is provided by the German Federal Ministry for Education and Research (BMBF) under grant no. 003X0131.

TH181

The role of epidemiology data in informing chemical risk assessment

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Preventing human harm from chemical exposures is fundamental to protect individuals and populations, and ensure the well-being of future generations. It is a foundational principle of public health protection. Nationally representative biomonitoring programs as well as smaller cohort studies have been tracking human exposure to environmental chemicals for many years. Some of the chemicals monitored for potential health effects are used in the manufacturing of plastics. Their uses are regulated based on risk assessments that largely rely on toxicity testing in animals, although many of these tests are considered outdated and not particularly driven by issues of public health concern. In contrast, epidemiological data continues to receive poor attention in regulatory decision-making. As currently performed, risk assessment relies primarily on animal data and chemical structure considerations to estimate a 'safe dose'; regulators consider this to be the amount of a chemical a person can be exposed to in a lifetime without causing harm. In the last two decades, mounting evidence has shown that some chemicals can cause adverse health effects at doses once considered safe. This is not uncommon for chemicals known to affect organ development or act as endocrine disruptors. Recent human data have shown that at least for some ortho-phthalates, members of a large family of plastic ingredients, early life exposures were associated with a number of health effects including delayed cognitive development, behavioural problems, advanced puberty and increased body weight. Other effects observed in children and adolescents were insulin resistance, increased blood pressure and disturbance in vascular function. We are currently testing the hypothesis that there is a discordance between the animal data used to determine safe levels of ortho-phthalates and the health effects associated with these chemicals observed in humans, a discordance that could potentially result in unsafe levels of exposure. We will discuss data showing that regulatory 'safe' levels of five ortho-phthalates (diisobutyl phthalate, dicyclohexyl phthalate, dibutyl phthalate, benzylbutyl phthalate and diethylhexyl phthalate) estimated using traditional toxicology testing protocols and endpoints may not be as health protective as once assumed, especially when exposures occur early in life.

TH182

Efficient and safe nutrient recovery from wastewaters

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The diminishing global phosphorus reserves and highly energy intensive nitrogen fertilizer production are inducing the need to seek for more sustainable sources of these nutrients. Recovering nutrients from a variety of industrial and societal side streams is becoming an important way to produce organic fertilizers. However, there are many constraints to reuse the recovered nutrients in a safe and efficient manner. In Finland, approximately 4,000 t of phosphorus and 27,000 tons of nitrogen are supplied to wastewater treatment plants annually. On average, approximately three quarters of the influent phosphorus remains in the final sludge, but is in a poorly soluble form and therefore unusable for plants in the short term. Only a fraction of nitrogen remains in the sludge, while most of it ends up in the air, effluent waters and reject water of the sludge drying/digestion. In 2016, 40% of sewage sludge was utilized in agriculture in Finland. Recently, the acceptability of sewage-based fertilizer products in agriculture has been questioned because of the remaining harmful substances and the risks and uncertainties related to them. These harmful substances include e.g. pharmaceuticals, flame retardants, surfactants and micro-plastics. Significant questions arise regarding the safety of sludge and wastewater based products. Although the effects of harmful substances on human health might be manageable, their effects on soil and biota are not sufficiently known. There are various technologies for recovering nutrients from wastewater. However, the introduction of new technology is expensive and the industry is not ready to invest if the guidelines are unclear. In NORMA-project (2019-2020, financially supported by Ministry of the Environment Finland), novel, innovative options to recover wastewater nutrients are evaluated. In order to create guidelines, a more holistic understanding of the possibilities to recover nutrients with different process technologies and their combinations is needed. The fate of harmful substances and the environmental and financial sustainability are important criteria influencing decision making. As a result of the project, various process alternatives and their pros and cons as well as possible data gaps are identified and presented in an informative way for decision makers and other stakeholders.

TH183

Applying an ecological framework to Yucheng rice oil poisoning incident: 40 years later

M. Li, National Taiwan University / Geography

In 1979, a rice oil mass poisoning occurred in central Taiwan. This epidemic was later referred to Yucheng and identified to be caused by the ingestion of commercial rice bran oil which had been accidentally contaminated with large amounts of polychlorinated biphenyls (PCBs) and their related compounds such as polychlorinated dibenzofurans (PCDFs). More than 2000 persons were identified as Yucheng victims. In fact, these Yucheng victims and offspring have still present health complaints or suffered from different symptoms up to the present. Although many epidemiological studies have been conducted for Yucheng, this incident hardly examined from the point of social-ecological framework. In past two decades, many health-related disciplines applied the ecological approach to understand different issues in disease prevention, health promotion and education. The ecological approach is a theory-based framework for understanding and exploring close relationships between individuals and society across multiple levels. By adopting the ecological framework as an analytical lens, this study will summarize and discuss epidemiological research of Yucheng and analyze society's responses to this incident through the individual, interpersonal, institutional, community, and public policy levels. At first, scientific studies published between 1979 and 2018 on Yucheng are summarized and updated to understand health effects of victims from PCB and PCDFs exposure. Next, historical newspapers are used as a tool to view how community and institution respond to this incident when Yucheng happened as well as to trace the historical development of this event over time. Finally, other sources of information, such as documentary films, magazines, unpublished theses or dissertations, will also include to address society's responses to this unfortunate incident at institutional, community, and public policy levels. Applying an ecological approach can help to provide a more holistic perspective of Yucheng incident. We shall emphasize the need to consider not just investigating health outcomes of victims from an epidemics event but also supporting with health care services for victims to reduce the consequences of an unfortunate incident. In sum, this review will discuss lessons learned from a food poisoning incident and how implementation of appropriate health policies to help victims and to prevent future poisoning incidents.

TH184

The trials and tribulations of working with Americamysis bahia: A mysid success story

M. Fox, Scymaris Ltd / Ecotoxicology; R. Maunder, Scymaris Ltd

The mysid shrimp *Americamysis bahia* has a reputation of being a difficult to species to work with; they are cannibalistic, easily stressed, prone to disease, can have variable fecundity and cultures can unexpectedly crash. Mysid shrimp studies are used in regulatory ecotoxicology in both the USA and Europe. In Europe, if a plant protection product (PPP) has an insecticidal mode of action (MOA) then the acute toxicity of an additional arthropod is required. This obligation can be satisfied by *A. bahia*, as well as any subsequent chronic testing if required. We decided to investigate the feasibility of mysid shrimp testing at our laboratory and after numerous challenges have recently started to reap the rewards of our hard work and determination. The main culturing challenge was the production of large numbers of healthy < 24 hour old mysids according to a pre-planned schedule for testing (140 and 420 for standard acute and chronic studies, respectively). We also solved various problems in husbandry methods and juvenile separation by developing novel in house procedures. We currently conduct flow-through acute and chronic studies to GLP as well as static high throughput screening tests. Each study design is carefully planned to incorporate the specific client needs, and can draw on a growing body of historic data to influence decisions on the required statistical power and experimental design. A recent challenging example is the requirement to use acetone as a carrier solvent to satisfy a client request that has led to several learning opportunities in very challenging testing conditions. This poster discusses the husbandry techniques and practical methods required to obtain a healthy, highly productive stock culture. We also show case studies of some of the interesting challenges faced and highlight our dedication to the novel problem solving required to produce a successful study.

TH185

SETAC Science and Risk Communication Interest Group

T. Seiler, RWTH Aachen University / Ecosystem Analysis

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Scientific Advancements towards Risk Assessments, their Frameworks and the Implementation of Alternative Strategies to Animal Testing for Nanomaterials (P)

TH186

Safety assessment of nanoparticles- A global regulatory perspective on data requirements

N. Rehman, Envigo Consulting Limited / CPC Consulting; S. Moore, Envigo

This poster will describe some of the key global regulatory frameworks e.g. EU REACH, US TSCA, FDA that are addressing the data requirements to demonstrate the safety of nanoparticles for both human health and the environment. Broadly speaking all these schemes are working towards a science and risk based approach. The use of nanotechnology is rapidly expanding in a wide range of household and personal care products such as clothes, electronic and cosmetics. Most of the current research is focused on carbon-based and metal oxide based nanoparticles e.g. nanosilver. In addition, there is huge potential in the use of nanotechnology to revolutionise healthcare in applications such as cancer treatment where nanoparticles are engineered so they are attracted to diseased cells only, which reduces damage to healthy cells in the body, allowing for earlier detection of disease. Regulatory schemes such as EU REACH Regulation, are developing specific requirements for the assessment of safety, with the new guidance expected to be published in Jan 2020. Key considerations for regulatory risk assessment i.e. characterisation, metrics and the fate/accumulation of nanoparticles in the environment will be discussed. The EU Inventory on Nanomaterials (EUON) also hosts health and safety information on nanomaterials on the EU market, and a recent study identified gaps in the hazard and risk assessment of 81 nanopigments. Under US TSCA, the EPA have assessed 160 nanomaterials and this includes a comparison of chemicals in their bulk and nanoform to understand the change in property causing an unreasonable risk. The US FDA also considers the effects of nanomaterials in the particular biological and mechanical context of an individual product and its intended use. For example, if the route of administration is inhalation, respiratory toxicity of nanomaterials can differ from larger particles, and OECD 412/413 Guidelines have recently been revised to incorporate this requirement. Data requirements for the FDA's pre-market review process data requirements for clinical and nonclinical studies will also be discussed.

TH187

Environmental safety assessment of nanomaterials: tools and needs

A.K. Jantunen, European Commission - Joint Research Centre / Directorate F Health, Consumers and Reference Materials; S. Gottardo, European Commission JRC; K. Rasmussen, Joint Research Centre / Nanobiosciences Unit

The NANoREG framework for the safety assessment of nanomaterials (2017) analysed how EU regulation, with focus on REACH, applies to nanomaterials, and discussed the availability and regulatory acceptance of approaches developed by NANoREG and others for addressing nanospecific issues. Since the publication of this report, the REACH annexes have been revised to contain specific information requirements concerning nanomaterials (to enter into force in 2020), the updating process of the ECHA guidance has started, and method development and standardisation for nanomaterials (e.g. by the OECD and various research projects) has continued. Progress has mainly been made on testing methods for physicochemical properties and human toxicity; the environmental side of nanosafety assessment is made difficult particularly by the complicated environmental fate phenomena and the monitoring of concentrations in complex environmental media. Using as inventory the contents of the NANoREG Toolbox for the safety assessment of nanomaterials (2017-2018), this poster will provide an overview of the regulatory requirements for the environmental safety assessment of nanomaterials under REACH and discuss how well the currently available and expected tools address these requirements.

TH188

EU Regulatory developments on the characterisation of nanoparticles

N. Rehman, Envigo Consulting Limited / CPC Consulting; D. Howes, Envigo Consulting Limited / Envigo Consulting Limited

Regulatory requirements for nanoparticles have been under development in EU, US and Canada. This includes data required on characterisation, and the safety of nanoparticles. Under EU REACH regulatory framework, the basic characterisation requirements for identity and composition for organic and inorganic substances are clearly described e.g. UV-VIS, X-ray diffraction etc. However, the requirements for nanoparticles have not been so well prescribed until very recently. In May 2017, ECHA provided a significant update in new guidance 'How to prepare registration dossiers that cover nanoforms: best practices', this included ; '*When the registrant manufactures or imports the substance in the nanoform as well as in the bulk form, the registration dossier should include the information of the substance in both the bulk form and nanoform'.* For some substances such as nanoparticles, other parameters in addition to chemical composition need to be considered in order to determine their impact on properties relevant for the hazard profile, and each registrant has to specify these parameters in their own dossier. Substances may be manufactured as nanomaterials and non-nanomaterials. For example, for a substance that is manufactured as a nanomaterial, there may be multiple nanomaterials that have a composition giving them the same substance identity, but different shape and surface chemistry. The three common elements for any nanomaterial are size, shape and surface chemistry, and these are the minimum requirements for data sharing and joint registration obligations. Nanomaterials may have different properties and thus different classification(s) for the relevant physicochemical, human health or environmental endpoint compared with the bulk (non nanoform) of the same substance. In addition, characterisation methods often require specialised equipment and expertise, and although nanomaterial manufacturers will have some expertise they may need to contract some of this analyses to specialist analytical laboratories. This poster will illustrate examples of the various nanoforms that could be obtained and the influence of characterisation i.e size, shape and surface chemistry on the hazard profile.

TH189

Colloidal stability and bioactivity of Fe3O4 nanoparticles functionalized with humics and silica

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Investigations of Fe_3O_4 nanoparticles (NPs) functionalized by different inorganic and organic ligands are being increasingly reported in the literature due to the importance for biomedical and ecological applications. The huge diversity of polymers allows for different types of Fe_3O_4 NPs surface functionalization, i.e., introduces charges on the system that can prevent the aggregation of the particles in liquids and improve their chemical stability through surface charge control. In addition to studying the size, structure and composition of the colloidal particles, control of their colloidal stability is very important. Controlling the stability of the magnetic fluids against aggregation is important because the formation of aggregates alters their specific surface area and dispersibility as well as their bioactivity. In the present work, the influence of humic acids (HA) and/or silanes (tetraethoxysilane, TEOS and 3-amino propyl-triethoxysilane, APTES) was studied and correlated with the colloidal stability and bioactivity. The control of the functionalization nature and density (number of layers) on the Fe_3O_4 surface was investigated, electrokinetic measurements (zeta-potential and hydrodynamic diameter), bioactivity analysis were performed. The higher plant − whitemustard *Sinapis alba* L., green algae *Scenedesmus quadricauda* (Turb.) Brev., and infusorians *P?ramecium caudatum* were used as test organisms for bioactivity evaluation. The results suggest that surface charge and bioactivity can be controlled by grafting Fe_3O_4 NPs with HA and/or silanes. The sequential layer-by-layer grafting of precursors onto the NPs surface demonstrated an logical increase in the hydrodynamic diameter (measured b? DLS) in the row: Fe_3O_4/TEOS < Fe_3O_4/TEOS/APTES < Fe_3O_4/TEOS/APTES/HA. Zeta potential for silica grafted NPs varied considerably with layering from -20 mV (Fe_3O_4/TEOS) to -0.6 mV (Fe_3O_4/TEOS/APTES), which correlates with surface charge provided by the surface amino group of APTES. For the Fe_3O_4 coated by HA zeta-potential increased from 15 mV (bare Fe_3O_4) to -40 mV (Fe_3O_4/HA). The bioactivity of bare and functionalized NPs by silica and/or HA with respect to test-organisms demonstrated higher toxicity for multilayered nanoparticles as Fe_3O_4/TEOS/APTES/HA in compare with Fe_3O_4/HA. *Acknowledgement.* This research has been financed by the Russian Foundation for Basic Research (*#18-33-01270/18)* and the Russian Science Foundation (#16-14-00167).

TH190

Suitability of Chlamydomonas reinhardtii and Chlorella vulgaris as test organism in a growth inhibition test according to OECD 201

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The OECD guideline for the testing of chemicals No. 201 describing the freshwater alga and cyanobacteria growth inhibition test recommends as green algae test species *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. To draw a comparison and potentially identify suitable candidates for SSD (species sensitivity distribution) testing, the green algae *Chlamydomonas reinhardtii* and *Chlorella vulgaris* were examined. The aim was to assess the growth of the two species under the conditions stipulated by the guideline and to compare the effect levels observed for the two species to the effect levels observed for *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*.

TH191

Graphene oxide ecotoxicity assessment using the amphibian Xenopus laevis: from toxicological effects to mitigation strategies

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Carbon-based nanoparticles such as graphene oxide (GO) possess unique properties triggering high expectations for development of new technological applications. This raises the question of potential adverse outcomes on living organisms and support the need to find innovative solutions to produce sustainable and safer nanomaterials for human and environmental health. This study focuses on the ecotoxicological assessment of GO towards tadpoles of the amphibian larvae (Xenopus laevis). The complete characterization of the tested materials as well as the measurement of multiple biological endpoints allowed to identify physico-chemical characteristics and toxicological pathways involved in the adverse effects observed on growth and genotoxic response. Larval growth inhibition was shown to be driven by GO surface area and metagenomics survey of gut bacterial communities was performed to understand the implication of adverse effects on gut microbiota in growth response. In addition, surface groups and toxicological pathway involved in the GO-related genotoxicity were determined through analysis of cellular and molecular responses. Thermal reduction of GO was shown to remove oxygen-containing functional involved in genotoxic response and may constitute a “safer-by-design” approach, allowing maintain of good processing ability, while avoiding the genotoxicity of GO.

TH192

Effects of ZnO nanoparticles on Algae, Daphnia and Zebrafish larvae

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The aquatic toxicity of metal oxide nanoparticles (NPs) is related to NP physicochemistry and the characteristics of the exposure medium. Toxicity of metal NPs has frequently been attributed to dissolution of metal ions; however, the contribution of particles to toxicity (or “nano-specific” effects) has not been resolved for most NPs. In this work, the toxicity of ZnO NPs (coated (152 nm) and uncoated (70-90 nm)) was studied, in parallel with the ionic $ZnSO_4$ in three different aquatic organisms: an algae (*Raphidocelis subcapitata*), a crustacean (*Daphnia magna*), and a vertebrate (zebrafish, *Danio rerio*). The toxicity of ZnO NPs in *R. subcapitata* was tested following OECD algal growth inhibition assay (guideline 201). Algal cells were exposed (96 h) to ZnO NPs (10 - 76 μg/L), and samples were extracted for chlorophyll analysis as a surrogate measure for algae population growth. Data indicates a decreased growth with increasing NP concentration. The daphnia acute immobilization test (48 h, following OECD test guideline 202) and chronic reproduction tests (21 days, OECD test guideline 211) were performed to determine the toxicity of the ZnO NPs and $ZnSO_4$. Results revealed an EC50 of 2.38 mg/L for the uncoated NPs, 3.07 mg/L for the coated ZnO NPs and 9.98 mg/L for $ZnSO_4$ after 48 hours. The chronic reproduction test (180-1400 μg ZnO/L) showed that reproduction decreased significantly in all treatment groups compared to the control group, and a higher mortality was observed in response to exposure to uncoated NPs compared to the coated version. The toxicity of ZnO NPs to zebrafish larvae was assessed following exposure (24 h) in two different media: 60 mg/L Seasalt and standard OECD media. Aqueous exposure media had a significant effect on the mortality of zebrafish larvae in resonse to ZnO NPs exposure. The LC50 for larvae exposed to ZnO NPs in Seasalt was 16.3 mg/L while only one larvae died in OECD medium. We show here that for both daphnia and zebrafish larvae there was no significant difference in the effects of the coated and uncoated ZnO NPs in the acute studies. However, chronic exposure to daphnids showed a higher mortality for the uncoated ZnO NPs. It is possible that release of Zn ions over time from the uncoated NPs contributed to chronic toxicity. Determining the contribution of ionic Zn and ZnO particles to induce lethal toxicity as well as the effect of test media in toxicity testing is critical for assessment of the toxicity of ZnO NPs.

TH193

The effect of short term exposition to silver nanoparticles and ions on testicle morphology and fertility of zebrafish (Danio rerio).

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One of the main places of deposition of nanoxenobiotics are aquatic ecosystems, however theirs toxicity, and especially the effect on fertility of aquatic organisms, has not yet been well-known. The aim of the work was to demonstrate the effect of nanoparticles and silver ions on zebrafish (*Danio rerio*) fertility and morphology of testicles. Fish at the age of 5 months were exposed on 0.01, 0.05, 0.1, 0.5, 1 and 2 mg/L of nanosilver solutions (AgNP group) or silver ions with concentrations of 0.01 and 0.05 mg/L (Ag + group). The duration of exposition was 7 days. Fish in control group were kept in conditions without xenobiotics. The fish were fed *Artemia salina* and commercial *ad libitum*. On the last day of the experiment the fish reproduction was applied according to zebrafish breeding procedure. After spawning, the fish were anaesthetized in MS222, fixed in Bouin's solution and then subjected to a standard histological procedures. Obtained histological slides were stained with the hematoksylin/eosin method to analyze morphology of the testicles. On the section across tubules in testicles the percentage of spermatogonia, spermatocytes and spermatids were calculated. After 6 hours incubation of eggs, the degree of fertilization was evaluated. On the last day of the experiment, statistically significantly lower survival was detected in the Ag+0.05 mg/L group, compared to the another experimental groups.

Microscopic analysis did not show histopathological changes in the structure of zebrafish testicles. In zebrafish males exposed to Ag + 0.05 mg/L, the percent of spermatocytes on the tubules cross section decreased, whereas the percent of spermatids significantly increased. It was observed that with the increase in the concentration of tested xenobiotics, the percentage of successful spawning decreased. However, no statistically significant effect of the studied xenobiotics on the degree of fertilization was found. The obtained results indicate that short-term exposure to a high concentration of the silver ions affects on spawning potential and the participation of various groups of germ cells in the parenchyma of testicles, which may lead to a reduction in breeding potential of zebrafish.

TH194

Cytotoxic effect of graphene oxide in rainbow trout monolayer cultures of primary hepatocytes without apparent cellular internalization

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Graphene oxide (GO) is commonly used in many industrial and commercial applications, consequently, their release into the aquatic systems is inevitable. Thus, aquatic organisms including fish can be affected by these potential pollutants. In order to determine their mechanisms of toxic actions, the use of in vitro approaches based on fish cells appear as a suitable tool. Of particular relevance are primary cultures of isolated fish hepatocytes, since they maintain similar functionalities as those of the original tissue. Taking all this into account, the objectives of this study were to investigate the potential toxic effects and intracellular fate of GO on primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Primary hepatocytes were exposed to a concentration range of 4-128 μg ml^{-1} of GO. Cytotoxicity of GO was determined on the same set of cells by means of the Alamar Blue (AB) assay (informing about alterations in the metabolic status of the cell) and the 5-carboxyfluorescein diacetate-acetoxymethyl ester (CFDA-AM, reflecting alterations in the plasma membrane integrity) assay following 3, 8, 24 and 72 h exposure. Additionally, the Neutral Red Uptake assay (informing about lysosomal membrane integrity) was applied after 72h exposure. GO caused cytotoxicity preferentially through alterations on cell membrane integrity after 3 h exposure at a concentration of 8 μg ml^{-1}. This cytotoxic effect was maintained at 8, 24, and 72 h. Reduced metabolic activity was detected only after 72 h exposure at 32 μg ml^{-1} or higher. However, GO did not affect lysosomal functionality at the tested concentrations. Transmission electron microscopy was used to further investigate the interaction of GO with cellular structures. Cells were exposed to GO at concentrations of 8, 16 and 32 μg ml^{-1} for up to 72 h. Interestingly, there were no signs of GO uptake so that intracellular GO could not be observed, and the cell membrane appeared to be intact. These findings are in accordance with the decrease of cell viability detected with the CFDA-AM and the AB assays (that reflect alterations in plasma membrane and metabolic activity) and the lack of effect in the NRU assay that would imply the internalization of GO. This research is supported by the EU's Horizon 2020 research and innovation programme (MSCA-IF-2016, Grant Agreement nº 746876)

TH195

Assessing human exposure risk and lung disease burden posed by airborne silver nanoparticles emission from consumer spray products

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Silver nanoparticles (AgNPs) have been applied to antimicrobial agent, disinfecting and deodorant spray products due to its high antimicrobial efficacy. However, lung adverse response may be induced by inhalation of aerosolized AgNPs while using spray products. Studies have proved the toxic effects of inhaled AgNPs. Related health risk was considered to exist in the application of AgNPs-containing spray products. The main aim of this study is to estimate potential human health risk of inhaled AgNPs by integrating the exposure analysis and rat-related effect data. A compartmentalized physiologically based alveolar deposition model was employed to estimate the lung AgNPs burden for long-term exposure in non-intensive and intensive applications. A Hill-based dose-response model was used to describe the relationship between the lung adverse effect and AgNPs accumulation. A Weibull threshold model was used to estimate the threshold dose of AgNPs causing 1%, 5%, and 10% increases of inflammation response. Moreover, we used the probabilistic risk model and hazard quotients (*HQ*s) to characterize the potential health risk. The results indicated that the total AgNPs lung burden could reach 0.87 mg from 2-year chronic exposures for an intensive application. The most conservative threshold accumulation amounts were 0.32 and 1.08 mg for exposing to 30 nm and 60 nm airborne AgNPs. In the most severe scenario, the probability of estimated *HQ*s being higher than 1 was 0.50. Based on levels of threshold accumulation amounts, we estimated the median threshold daily exposure durations (ranging from 2.5 – 22.3 h d^{-1}) as recommendation for occupational workers or consumers using AgNPs-containing sprays. We conclude that intensive application of spray products under long-term exposure is likely to pose higher risk on human health. We also provide the threshold daily exposure duration as recommendation for users of AgNPs-containing spray products. This study provides a mechanistic approach on estimating potential health risk for the long-term exposure to airborne AgNPs from spray products. *Keywords*: Silver nanoparticles; Spray product; Physiologically based alveolar deposition model; Risk assessment

Incorporating Ecosystem Functioning into Environmental Quality Assessment (P)

TH196

Assessment of chemical risk to ecosystem services: a proof of concept study

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Ecosystem services are the direct and indirect contributions that ecosystems make to human well-being. The ecosystem services concept is gaining broad interest in regulatory and policy groups for use in landscape management and ecological risk assessment (ERA). A recent multi-stakeholder evaluation of the use of an ecosystem services approach in guiding chemical environmental risk assessment has identified clear advantages of using the approach, but also highlighted a number of challenges related to its implementation[1,2]. This proof-of-concept project evaluates the feasibility of adopting an ecosystem services approach to chemical risk assessment using current knowledge and tools. The outcomes of this project will inform research and regulatory agendas. The project is focussed on three regulatory-relevant case studies: prospective ERA for plant protection products (Regulation 1107/2009 EC), prospective ERA for general chemicals (REACH) and a retrospective ERA for aquatic systems (Water Framework Directive). The rationale, approach and initial findings are presented. [1]Maltby et al 2018. Sci Total Environ 621:1342-1351; [2]Faber et al 2018 Sci Total Environ 651: 1067-1077

TH197

Functional endpoints as new perspectives for the definition of protection goals in regulatory ecotoxicology

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Currently the development of guidance documents by EFSA for regulatory ecotoxicology under regulation (EC) 1107/2009 is lacking by the definition of specific protection goals. To this aim, we provide data comprising a comprehensive ecotoxicological data set highlighting the importance to use structural and functional endpoints to describe and understand ecological entities. Specifically, data of freshwater nematodes and other sediment-dwelling organisms, based on long-term indoor microcosm studies with metals and PAHs as model pollutants, is provided on the typical structural parameters abundance, diversity, and biomass (B) as well as on the functional parameters secondary production (P) and its ratio to biomass P/B. The concurrent investigation of an extensive range of nematode species in the context of a complex benthic food web gives insight into general ecosystem functioning. The data shows sensitivities of different endpoints and gives a hint to mutual dependencies.

TH198

Biochemical characterization of six economic valuable marine bivalve species from the Portuguese coast

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Marine bivalves are distributed worldwide through diverse habitats, including estuaries andcoastal lagoons, and play important roles in the trophic webs, as they can connect primary producers to consumers, and are able to control several ecosystem' processes, like the oxygen penetration in the sediments. As well as other seafood, bivalves are highly appreciated by humans and represent an important economic value. Consequently, marine bivalves are under pressure due to an increasing demand for seafood resources. Aquaculture production can be an approach to overcome this demand. Aquaculture development has a stronger manifestation in some Asiatic, American and European countries, like Portugal. In the present study, six commercially valuable species of marine bivalves (*Cerastoderma edule*, *Crassostrea gigas*, *Mytilus galloprovincialis*, *Ruditapes* *decussatus*, *Scrobicularia plana* and *Solen marginatus*) were sampled in two distinct areas of harvest and aquaculture production in Portugal, the Mondego estuary and the Ria Formosa lagoon, and in two seasons, winter 2016 and summer 2017, in order to 1) determine the biochemical composition in terms of total protein content, fatty acid and carbohydrate profiles, 2) identify potential spatial and seasonal variations in the biochemical composition and 3) assess feeding behaviour of the bivalve species in both seasons and study areas. All species demonstrated higher total protein content, followed by diverse fatty acid content, specially DHA and EPA, and glucose and glycogen as the main neutral sugar and polysaccharide, respectively. A tendency for omnivory was confirmed in all bivalve species, with only *S. marginatus* presenting clear herbivorous behaviour in summer. *M. galloprovincialis* and *R. decussatus* showed the highest nutritional value in the Mondego estuary, in both seasons, although more noticeable in winter. In Ria Formosa, *C. edule* and *R. decussatus* presented the highest nutritional value in both seasons, while *C. gigas* exhibited higher nutritive value in summer. The bivalve species with higher nutritional value are suggested, in this study, as best suited to be produced in aquaculturein each area, respectively. This study was supported by Fundação para a Ciência e a Tecnologia (FCT) through the strategic projects UID/AMB/50017/2013 granted to CESAM and UID/MAR/04292/2013 granted to MARE. FCT funded A. M. M. Gonçalves and C. Nunes (SFRH/BPD/97210/2013 and SFRH/BPD/100627/2014).

TH199

River bacteria community analysis by flow cytometry as bioindicator of Water Quality

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Freshwater ecosystems are suffering enormous pressure from anthropogenic activities, such as agriculture, industry and climate changes, leading to its degradation. The purpose of the Water Framework Directive (WFD) implementation within European Union members, in 2000, was to regulate all water bodies reaching at least good ecological status in all water bodies. The major WFD disadvantages is being costly and time-consuming. In order to address that disadvantages the objective of this study was to use the scoring provided by flow cytometry analysis (FCM) of river bacterial communities into high DNA content (HNA) and low DNA content (LNA), a fast and easy screening methodology, as bioindicators. Caima river was used as case study in 3 sampling sites with different levels of environmental impact (site 1- no anthropogenic impacted site; site 2- downstream wastewater treatment plant and site 3- downstream a deactivated mine) were analysed along the 4 seasons of the year 2017 (winter, spring, summer and autumn). The ecological status evaluation of the sampling sites was assessed following the methodology described by WFD. The results obtained for macroinvertebrates and periphyton communities in terms of standard biotic indices as well as biological oxygen demand (BOD_5), were compared with the bacterial HNA and LNA content of the samples. FCM data analysis showed high bacteria density at site 2 associating with high amounts of organic input by a wastewater treatment plant (WWTP). Also, HNA bacteria were found to be in higher quantity at site 2, related to the increase of nutrients, while LNA bacteria were present in higher density in river headwaters (site 1), corresponding to an oligotrophic environment. Correlations between biological indices and bacteria community composition were very strong, showing that bacteria communities may serve as bioindicators of water quality assessment pending further investigation considering a wider array of samples.

TH200

Planktonic vs benthic community sensitivity towards 3,5-dichlorophenol, potassium dichromate and lead chloride

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Biological diversity represents a major challenge in ecotoxicological assessment, since each species will respond differently to similar levels of exposure to toxic substances. By including in this assessment approach organisms which are representative from different functional groups, one theoretically captures the community range of variability regarding toxicant sensitivity. The variation of the sensitivity of species within a community or an assemblage towards one or more toxicants can be expressed in terms of cumulative distributions known as Species Sensitivity Distributions (SSDs). SSDs have been increasingly employed in the determination of chemical concentration benchmarks that represent protective levels for most species in the environment. Besides this practical application, SSDs could be an interesting tool to compare the sensitivity of several freshwater organisms, positioning them in a curve according to a sensitivity rank. This is the arena for the development of the present study, where the representativeness of standard organisms widely used to build SSDs was particularly challenged on whether they reflect the responses of benthic communities in lotic ecosystems. SSD are constructed mostly based on ecotoxicological tests with standard species that typically represent lentic freshwater ecosystems, and their use for environmental hazard in lotic ecosystems is somehow limited since planktonic species have no expression in these systems. The comparison of HC_5 and HC_{50} for each SSD curve, and for each community and chemical tested, allowed us to conclude that SSD built based on planktonic organisms might not be protective enough to benthic organisms, and consequently for lotic environments. Furthermore, benthic diatoms showed to be the most sensitive organisms to all chemicals tested evidencing that this specific community might be at risk if typical benchmarks based on testing with standard species are used for regulation.

TH201

Size spectra analysis reveals structural changes of natural communities under realistic exposures to pharmaceuticals and personal care products

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The extent of freshwaters contamination by a multitude of bioactive organic pollutants collectively classified as pharmaceuticals and personal care (PPCPs) products calls for concern. Ecological assessments of the burden that PPCPs might impose on natural aquatic ecosystems are still scarce. In a field experiment, we tested whether a realistic single time exposure to mixture of 12 pharmaceuticals and personal care products, commonly found in European inland waters, can influence the size structure of natural lake phytoplankton communities. Size is generally considered as one of the most influential determinants of community structure and functioning. Using an *in-situ* microcosm approach, phytoplankton communities in two lakes with different nutrient levels (mesotrophic and eutrophic) were exposed to a concentration gradient of the PPCPs model mixture at five levels. We tested whether sub-lethal PPCP doses can affect the slope of size spectra, which describe changes in the abundances of small compared to larger phytoplankton. Size spectra was drawn from a power-law distribution. PPCPs were found to induce changes in the size distribution of natural phytoplankton communities in both lakes. The two highest treatment levels of PPCPs were found to consistently decrease the abundance of the most dominant size class (nano-phytoplankton: 2-5 μm). The slope of size spectra became shallower with increasing PPCPs concentrations reflecting the decline in the density of small phytoplankton. These results strongly indicate that environmentally realistic exposure to PPCPs can have cascading effects in the aquatic food webs by favoring the larger non-edible algae at the expense of small edible species.

TH202

Artificial algal communities under Cu stress: effect of functional diversity on biomass production

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Standard toxicological testing with single species does not capture the complexity of toxicant effects in natural ecosystems. We aimed to narrow this gap by measuring how functional diversity (FD) affects primary biomass production - an important ecosystem function - of artificial microalgal communities at different levels of algicidal toxicant stress. In a modified OECD 201 test, we evaluated the biomass yields of all 15 single- and multi-species combinations of four functionally different algal species (green algae *Chlamydomonas reinhardtii* and *Raphidocelis subcapitata*, diatom *Fistulifera pelliculosa* and cyanobacterium *Synechocystis sp*) after a 72-hour incubation with 0, 0.01 and 0.02 mg Cu/l in triplicate. FD of the communities was assessed by community-weighted means (CWMs) of 10 algal functional traits and mean pairwise distance (MPD) calculated based on the traits. Overall, biomass yield significantly decreased at 0.02 mg Cu/l compared to lower toxicant levels. Significantly lower biomass ($p< 0.001$) at higher levels of MPD was observed at 0 and 0.01 mg Cu/l, while no effect was detected at 0.02 mg Cu/l, indicating that higher FD did not lead to more biomass production. However, because the four tested species are functionally distinct, species richness (SR) can be used as a proxy for FD. SR had a significant positive effect ($p< 0.01$) on biomass yield at each toxicant level, which may be explained by highly productive species in our selection being represented more often in multi-species communities. CWMs of two size-related traits, greatest linear dimension and logarithm of cell size, caused a significant ($p< 0.01$) increase in biomass yield at every toxicant level. Binary trait (motility, colony-forming, pigment content, Si content) CWMs also significantly affected biomass yield, but they were mostly intrinsically related to one species, impeding the interpretation of the effect. In conclusion, we observed a positive effect of SR and cell size on algal community biomass at all Cu concentrations, but a negative biomass effect of the functional diversity index MPD at 0 and 0.01 mg Cu/l. Our novel experimental approach allows for more environmentally relevant toxicity testing that reveals patterns in ecosystem functioning in response to toxicant stress, while retaining the advantage of being conducted in controlled laboratory conditions.

TH203

Biomonitoring of phytoplankton communities changes following heavy metals fluctuations in lake Balkhash ecosystem

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In the world threatened by multiple anthropogenic impacts, innovative approaches to lake ecosystems monitoring and assessment are required for proper water management. We have designed an innovative approach combining the characterization and counting of microalgae groups based on imaging flow cytometry (IFC; FlowCam) coupled with detailed chemical analysis. The aims of our multi-parametric approach are to monitor the lake waters for phytoplankton diversity, composition, abundances, and correlate it with physico-chemical parameters and heavy metals concentrations. We performed biomonitoring of 14 sampling stations at Lake Balkhash, one of larges lakes in Asia located in southeastern Kazakhstan. The shallowing of Balkhash lake and environmental impacts of emissions due to mining, metallurgical processes, and inflow of polluted water from Ili river (comes from China) creates serious concerns about the functioning of the lake ecosystem. In details, IFC biomonitoring of phytoplankton communities was performed in parallel with chemical analysis of surface water for heavy metals (Fe, Cu, Zn, Mn, Cd) and ions (potassium, ammonium, nitrates/nitrites) by atomic absorption spectrometry with Analyst 400 (Perkin-Elmer) and ion chromatography with Methron Compact IC FLEX 930 (Methron). Our characterization of morphological traits of microalgal cells by IFC offered a high level of statistical reproducibility and allowed to characterize the response of phytoplankton communities to applied stressors such as fluctuations in heavy metals concentrations and ions. RDA and bivariate correlation analysis demonstrated the positive correlation of nanoplankton abundance with increased levels of Fe (r=0.83) and large pennate diatoms (including *Ulnaria sp* and *Entomoneis sp*) with manganese and copper. Identification of microalgal groups that may serve as bioindicators of changes in phytoplankton communities structure and diversity towards heavy metals and other pollutants is expected to lead to better monitoring and environmental management of lake aquatic biosystems. The poster presents methods and results with a major focus on multi-parametric innovative approach and a correlative analysis of heavy metals levels and phytoplankton taxonomic group abundances and diversity.

TH204

Soil microbial activity in relation with stages of plant colonization in mine tailings soils of a Mediterranean area

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Mine tailings show high metal levels, extreme pHs, limited organic matter and deficient physical properties. As a consequence soil biota activity and biogeochemical cycling might be hindered, affecting soil functioning. Most tailings are abandoned without any restoration and might be spontaneously colonized by plants. This colonization occurs along with changes in functional soil properties. Identifying these changes helps to understand those processes underlying natural ecosystems recovery, and might be used to design more effective management for reducing tailings environmental risks. This study aims to assess soil microbial activity in mine tailings soils related to different stages of spontaneous colonization by native vegetation under Mediterranean climate. Six environments were studied in an abandoned mining area (SE Spain, n=4): A) Within mine tailings: 1. Bare soils (S); 2. Small groups of *Pinus halepensis* trees (2-5) ≤≈2.5 m high growing scattered (P); 3. Isolated *P. halepensis* trees >≈4 m high with shrubs and herbs under the canopy (P+MS); 4. Dense patches with several *P. halepensis* trees (>5) >≈4 m high and shrubs and herbs under the canopy (DP+MS). B) Outside mine tailings: 5. Contaminated forest with *P. halepensis* trees >5 m high and shrubs and herbs under the canopy (PF); 6. Non-contaminated control forest with *P. halepensis* trees >5 m high and shrubs and herbs under the canopy (CF). Soil microbial activity (upper 15 cm) was assessed by quantifying microbial biomass carbon (MBC), dehydrogenase/β-glucosidase activities and soil respiration. CF showed the highest MBC, β-glucosidase activity and soil respiration (1523 mg C kg^{-1}, 3.76 μmol p-NP g^{-1} h^{-1} and 0.74 g m^{-2} h^{-1}, respectively) which could be related to less stressful conditions in non-contaminated forest soils. However, inside mine tailings, resources scarcity could have led to lower microbial activity (e.g., 170 mg C kg^{-1}, 1.09 μmol p-NP g^{-1} h^{-1} and 0.34 g m^{-2} h^{-1} in DP+MS). P showed the highest dehydrogenase activity (4.75 μg INTF g^{-1} h^{-1}) which could be related to the presence of some adapted functional groups. CF also showed high dehydrogenase activity (3.28 μg INTF g^{-1} h^{-1}), while it was lower in the other stressed environments (0.02 – 2.66 μg INTF g^{-1} h^{-1}). The characteristics observed in the different environments showed that it could exist functional differences among them. Soil microbial activity was closer to CF in those tailing environments with higher plant colonization.

TH205

SETAC Salinization of Freshwater Interest Group

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TH206

Effects of imidacloprid exposure on reproduction and survival of two Collembolan species

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Exposure of non-target organisms to pesticides may have serious ecological implications. To address this issue, we used our recently-developed experimental framework to compare how reproduction and survival of two different soil community species (Folsomia quadrioculata and Hypogastrura viatica) of the keystone arthropod order, Collembola, are affected by dietary exposure to the pesticide, imidacloprid, under different exposure scenarios. We focused on comparing the responses of these two species across selected doses of imidacloprid. The food (cyanobacteria growing on the bark of a tree in the campus of the University of Oslo) was spiked by scraping off the cyanobacteria and soaking in 0.429 and 4.29 ng mL^{-1} aqueous solutions of imidacloprid (equivalent to imidacloprid concentrations of 0.01 and 0.1 mg kg^{-1} dry soil) overnight, followed by filtration. The dried filters covered with the cyanobacteria were offered to the Collembolans. All the experimental replicates and controls started with 20 adult Collembolans, and the exposure lasted for 14 days at 15°C. Thereafter, the animals were reared in a clean environment for 25 days. Cumulative egg production per animal and survival, recorded throughout the rest of the experiment, were compared. The effects of imidacloprid exposure, species, and exposure x species interaction on reproduction and survival were compared. We expected that imidacloprid exposure as well as the responses of the two species would be significantly different. Specifically, we expected that F. quadrioculata to be more sensitive than H. viatica. The findings would provide the foundation for more detailed studies on the effects of pesticide residues in soil Collembola communities.

Poster Corner Abstracts

Micro(Nano)plastic Pollution: Tackling the Plastic Problem by Identifying Sources, Investigating Fate and Novel Approaches (PC)

MOPC01
Microplastic contamination in bivalves from the Korean Coast
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Microplastic contamination in the marine environment is an increasing concern. Bivalves are useful bioindicators of microplastic contamination in the marine environment due to several advantages such as extensive filter feeding activity and broad geographical distriburion. This study investigated microplastic contamination in bivalves at 12 sites along the Korean coasts. The concentration of microplastics in bivalves are in range of 0.05 – 1.27 n/g (0.20 – 6.68 n/individual). The major polymer type was polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyester (PES). The fragment was dominant shape, and the most common size class was 100 – 200 μm.

MOPC02
Microplastics in environmental samples of Tarragona coastal area
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Microplastics (MPs), plastic particles

MOPC03
Analysis of Microplastics in German Drinking Water
Y.K. Müller, M. Pittroff, C.S. Witzig, N. Zumbülte, TZW- DVGW German Water Centre / Department of Water Chemistry Research
As microplastics have been found in many environmental matrices like lakes and rivers, the question arises whether microplastics can also be transferred into drinking water that is derived from these raw water sources. In this study, a fractionated filtration unit with stainless steel filters sieves (100 μm, 20 μm, 5 μm mesh size) was developed and validated (recovery rate of 81%) for the sampling of microplastic particles in drinking water. A sample volume of 0.3-1.3 m^3 processed drinking water was sampled in three German waterworks, which use different raw water sources (groundwater or surface water), and one tap water respectively. After an extraction of the filter sieves in an ultrasonic bath and subsequent vacuum filtration over a PTFE filter membrane, all particles with diameters of 5 μm and above were analysed with micro-Raman spectroscopy. For all drinking water and tap water samples, the microplastic content (180-801 microplastic particles per m^3) was in the range of the process blank values (ranging from 51-531 particles per m^3). Primarily polyethylene (PE) and polyoxymethylene (POM, part of the filtration unit) were found in the samples, besides polypropylene (PP), poylamide (PA), polyethyleneterephthalate (PET) and polyvinylchloride (PVC). An investigation of blank values is crucial for an assessment and evaluation of microplastic data, this is particularly important in matrices that contain very low numbers of microplastic. Thus process blank values were done for each sample and measures were taken to avoid contamination during sampling, sample preparation and analysis. The number of microplastics strongly increased in the lower micrometre range below 20 μm (76% of all microplastics). This observation stresses the necessity to sample and analyse particularly microplastics in the lower micrometre range due to their high numbers and potential toxicological relevance. Whereas first results indicate very low numbers of microplastics in drinking water, further research and method optimization is crucial to get a holistic overview about microplastics in drinking water.

MOPC04
High levels of microplastics on exposed rocky beaches ; hotspots that allow for compositional comparisons, while introducing new exposure data regarding environmentally relevant concentrations
T.M. Karlsson, M. Threapleton, University of Gothenburg; M. Hassellöv, University of Gothenburg / Department of Marine Sciences
Researchers have analyzed surface waters and sediments, from all over the world, for the presence of microplastics (MPs). The findings show that MPs are ubiquitous in the marine environment. However, the low number of particles in most samples, in combination with high variations between replicates complicates compositional comparisons. Floating plastic debris is known to accumulate outside the Swedish west coast as they are transported from both the North Sea and the Baltic with surface currents. This debris also strand on the exposed beaches of the archipelago, especially following stronger winds and waves. Through modelling and field experiments with drifters it has also been shown that local sources are likely to strand along the coastline of the inner part of the archipelago, at least temporarily. Here a screening of three exposed beaches and three beaches outside coastal urban areas was performed using pooled samples over a 3 m transect and a 'worst case scenario' sample where plastics were seen to accumulate. The beach characteristics differed significantly between the different locations, complicating direct comparisons. Initial analysis however shows that with more than 1700 microplastics >300 μm per g sample, the most exposed beach had ten times the average concentrations for the urban beaches. These high numbers also show that exposed rocky beaches can accumulate microplastics, providing new data on exposure levels at microplastic hotspots. In addition the high number or particles per sample, also for the urban samples, allows for discussions on relative compositions of microplastics.

MOPC05
Fate of microplastics at Nenäinniemi wastewater treatment plant, with special emphasis on discfilter -based tertiary treatment
K. Ryymin, P. Salmi, University of Jyvaskyla / Department of Biological and Environmental Science; A.K. Karjalainen, FCG Design and Engineering Ltd / FCG Finnish Consulting Group Ltd; A. Mikola, Aalto University / Department of Built Environment; J. Talvitie, Finnish Environment Institute / Marine management
Wastewater treatment plants (WWTPs) equipped with secondary treatment systems have been shown to remove up to 99% of microplastics (MPs, polymer particles < 5 mm). However, considerable amount of MPs are still released within the final effluent. This study was conducted on the Nenäinniemi WWTP in Central Finland, where we aimed to quantify the MP retaining capacity of an advanced discfilter -based tertiary treatment. Samples taken after each treatment from influent to dried sludge and final effluent were purified using newly established purification protocols developed especially for MPs. Purified microplastics down to 20 μm in diameter were photographed, counted and their chemical composition analyzed using micro-raman spectroscopy. Finally, a comprehensive analysis of the WWTP's microplastics balance was done to determine removal rate and the fate of MPs in the Nenäinniemi WWTP. Our results demonstrated that the discfilter was efficient, although not explicit, setup to remove microplastics from wastewater. We also report empirical modifications on the state-of-the-art MP purification protocols that facilitate purification of samples with high content of solids. \n

MOPC06
Spatial Study of Microplastic Pollution in the Mississippi River Basin
J. Cizdziel, University of Mississippi / Chemistry; A. Scircle, Z. Gao, University of Mississippi
Microplastic (MP) concentrations along the northern Gulf of Mexico are among the highest levels reported globally. The most likely source of the particles is the Mississippi River (MR) which drains much of the central portion of the United States. Yet, surprisingly little is known about the concentrations, types, sizes, and loadings of MPs in the MR and its major tributaries. This lack of data is hindering our understanding of the magnitude and sources of the problem. Because the Mississippi River is an intricate system of waterways, tributaries, and commercial routes, an in-depth spatial study is needed to fully assess MP pollution in the system. Our research aims to systematically quantify the concentrations and loads of MPs and characterize their shapes, size distribution, and chemical composition in the MR system—a source of drinking water to over 18 million people. To that end, we used nile red staining and subsequent fluorescence microscopy for MP counting and vibrational spectroscopy to confirm the identity of plastics found in samples. The morphology of the MPs was dominated by fibers (~75%), followed by fragments (~23%) and beads (~2%), with the proportion of fragments increasing slightly moving down the main stem of the river. The concentration of MPs is lower for small tributary rivers (Tennessee and Yazoo) than in the MR; however, some larger tributaries (Ohio and Missouri) have higher concentrations than the MR at the area where they converge. This is important to assess possible relations between MPs levels and characteristics with sources and different watershed attributes. Counts and loads of MPs generally increased down the main stem of the MR until past New Orleans, where loads declined, possibly due to deposition with slowing water. Sites near population centers (e.g. Memphis) had higher MP concentrations. Finally, we will report on a "one pot" method where MP samples can be collected, stored, digested, density separated, and stained to minimize contamination and avoid transfer losses.

LCA Applications and Critical Methodological Developments for Sustainable Nutrition (PC)

MOPC07
Environmental impacts of food: variability and mitigation potentials through producers and consumers

T. Nemecek, Agroscope / Agroecology and Environment; J. Poore, University of Oxford / School of Geography and the Environment
Global food production is a major driver of environmental impacts. Today's food system occupies 38% of the desert- and ice-free land, causes 26% of greenhouse gas (GHG) emissions, 32% of terrestrial acidification, 61% of freshwater withdrawals, and 78% of eutrophication. To keep environmental impacts within the platenary boundaries, drastic changes in food production and consumption are needed. The present study carried out a meta-analysis of 570 studies, with data from 119 countries. A harmonised database was derived for five environmental indicators: land use (land occupation), freshwater withdrawals (and scarcity-weighted freshwater withdrawals), global warming, acidification and eutrophication potentials. The data were standardised in several steps by: correcting differences in functional units, emission factors, characterisation factors, allocation methods, and system boundaries. Missing life cycle phases were filled by standard data. Emissions and environmental impacts were recalculated, whenever needed. The global totals were found to be consistent with global estimates. The meta-analysis of food LCA studies showed that large variability exists between producers of the same product, indicating substantial mitigation opportunities. The 90th percentile impacts were between 4-11x higher than the 10th percentile impacts for global warming, land use, acidification and eutrophication and even 5500x higher for scarcity-weighted water use. The impact distributions are hightly skewed, with 25% of the producers causing about half of the environmental impacts. We found that different producers require different ways to reduce their impacts; no universal solutions exist. To define a mitigation strategy a detailed analysis of each production system in its context is therefore indispensable. Furthermore, trade-offs between different environmental impacts have to be taken into account. Consumers can mitigate environmental impacts by reducing their consumption of animal-based food and by avoiding producers with high environmental impacts, with synergies between these two strategies. To achieve these improvements, better information on the environmental impacts must be made available and communicated along the value chain. Reference: Poore J, Nemecek T. 2018. Reducing food's environmental impacts through producers and consumers. Science 360, 987-998.

MOPC08

Defining sustainable food products by integration of nutrition to food LCA

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Introduction of nutritional values to the food LCA appears necessary when sustainable food products are aimed to be defined. The method should be related to a scope and goal of the study, as all methodological solutions in an LCA. Accordingly, validity of the methods is related to the purpose of the study. This study aims to evaluate an introduction of nutrition to the food LCA from the point of view of consumer education towards sustainability. Building on recent developments in the field (Saarinen et al. 2017; Stylianou et al. 2017) this study presents a framework and a stepwise process to evaluate and define sustainable food products including environmental and nutritional aspects in the assessment. The positive product-group-specific nutrient indexes are suggested to be used as a baseline functional unit (FU) in an integrated food LCA. Mass-based FUs for individual essential nutrients specific to a product group in question is suggested to be used as complementary FUs. Alongside these measures a reference flow (RF) for a nutrient indexes based FU and a reference amount (RA) for Daily Recommended Intake of individual essential nutrients are suggested to be utilised as a mean to take an effect of a portion size into account in a process of defining sustainable food products. Nutrients harmful to health in typical portions are suggested to include in an assessment as a separate LIM index based on harmful nutrients or as an impact indicator based on adverse impact of harmful nutrients on human health. The procedure is demonstrated by an assessment of case-products from the product groups of protein sources.

MOPC09

GHG emissions and nutritional quality of home cooked meals: a fair comparison.

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Abstract To date, many studies have assessed the environmental impacts of food products or diets, using different methodologies or approaches. However, few of them have considered their nutritional quality. This study aims to evaluate, on the one hand, the greenhouse gases (GHG) emissions of four home cooked meals composed of multiple ingredients, through a Life Cycle Assessment (LCA), and, on the other hand, to link the GHG emissions to the nutritional qualities of those meals. The selected meals suit different types of diets: vegetarian, carnivorous, pescetarian and omnivorous. Those meals are homemade according to traditional recipes, and different ways of cooking the ingredients (pan fried, boiled, oven baked) are taken into account. The LCA of the meals is performed from cradle to consumer, including all the relevant stages of their life cycle: food production, manufacturing, distribution, cooking and consumption. The nutritional quality of the meals is assessed using the nutrient rich food index 9.3 (NRF9.3), which takes in account 9 nutrients to encourage (protein, fiber, Vitamins A, C and E, Ca, Fe, Mg, K) and 3 nutrients to limit (saturated fat, added sugar, Na). To compare the GHG emissions of the meals, three functional units are considered: one meal adjusted by grams, kcal, and NRF9.3. The study aims to discuss the importance of introducing the nutritional quality in LCA when comparing the environmental impact of meals, in order to go beyond the traditional results based on mass or energy intake only, as mainly found in the literature. **Acknowledgement** This research study is carried out as part of the Ceres-Procon Project: Food production and consumption strategies for climate change mitigation (CTM2016-76176-C2-1-R) (AEI/FEDER, UE), financed by the Spanish Ministry of Economy and Competitiveness. The authors are responsible for the choice and presentation of information contained in this paper as well as for the opinions expressed therein, which are not necessarily those of UNESCO and do not commit this Organization.

MOPC10

Incorporating health and nutritional quality of food in LCA to create sustainable and healthy meals for catering

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Our current way of food production and consumption is not only one of the major sources of environmental impacts but is also responsible for a number of health challenges we face today that are directly linked to global dietary transition and the food we eat. As the frequency of eating out increases, catering has a great potential to lead the transition to more sustainable food systems by offering healthy and sustainable meals. Life Cycle Assessment (LCA) can serve as a useful tool for assessing the environmental impact of menus and for creating low environmental impact meals. However, LCA studies on catering and menus often fail to incorporate the health dimensions of meals, which are equally important, if we are to create meals that are able to safeguard both human and planetary health. Without the inclusion of health and nutritional quality of food in LCA, we are risking the chance of creating meals that may be more sustainable from an environmental point of view (e.g. have lower GHG emissions and environmental impacts) but would still not promote the most optimal dietary pattern for health. The aim of this paper is to contribute to the development of a set of tools and criteria that can used in the LCA process to assess not only the environmental impacts of different meal options but also their health implications. This will help us to maximise the health benefits of certain food items (e.g. unprocessed whole foods) and create meals that not only have low environmental impacts but are also naturally abundant in health-promoting properties. This study employs desk-based research and reviews existing dietary, nutrient and food-based guidelines and UK government dietary recommendations for public institution catering, and compares them with the latest, evidence-based scientific literature on what is considered a healthy diet. A particular focus is on how the latest scientific evidence on the most healthful food groups and dietary patterns can be incorporated into the LCA process in order to increase the robustness of LCA in helping us identify options that yield truly sustainable outcomes. Building on that, a set of criteria on health and nutritional quality is identified which can be used as a tool alongside LCA to evaluate the health outcomes of different meal options.

MOPC11

How to integrate nutritional recommendations and environmental targets into food labels: a university canteen example

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Certain shifts in dietary patterns are leading to an increased incidence of noncommunicable diseases such as diabetes and heart diseases. About 30% of the total resource consumption of EU-citizens is related to food consumption. In addition, the nutritional value and environmental impact of food is not always clear to consumers. For this reason, promoting healthy and environmentally friendly meals are of vital essence. To decide what these meals are**,** environmental targets and nutritional recommendations can be adopted to classify meals. The aim is to classify meals based on environmental policies and nutritional recommendations in function of the proposition of a food label. To achieve this goal, we quantified the environmental impact and nutritional value of meals. As a case study, we analyzed hot meals served in the canteen of Ghent University. Every meal contained three meal components: a protein, vegetable, and carbohydrate component. Every day three or four different protein, two vegetable, and five carbohydrate components were served. Consumers could chose one of each meal component. All meal combinations of three consecutive days were evaluated, i.e. 100 meals in total. The main groups of meals were ruminant meat, non-ruminant meat, fish, and vegetarian meals. To classify the meals, we introduced three threshold values for both environmental impact and nutritional value. This corresponds to four environmental and four nutritional classes which results in 16 classes in total. The environmental and nutritional threshold values were based on the 2020 European target on reduced emissions of greenhouse gasses (EC, 2018) and the Flemish food recommendation (Vlaams Instituut voor

Gezondheidspromotie en Ziektepreventie, 2012), respectively. We translated the results of the classification in a label, which communicates the score of a meal and why a meal did not get the best score by mentioning the hot spots (Cooreman-Algoed et al., in preparation). The main conclusions are that meals with fish had the best overall score and meals with ruminant meat the worst score. Vegetarian meals had the best environmental and, similar to ruminant meat meals, the worst nutritional score. The environmental hot spots were, in descending order in terms of magnitude, ruminant meat, deep-fried products, meat portion, greenhouse vegetables, and rice. The nutritional hot spots were, from most to least prevalent, total fat, salt, calories, and saturated fat.

MOPC12

Life cycle indicators for sustainability in Food Chain Practices and Diets

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The food sector has become the subject of increasing attention as they are among the production chains with the greatest environmental impact. the object of more international companies is pushing food systems to converge towards models of sustainability and consumers to a healthier and balanced diet. In particulary, the factors linked to food sustainability find in the diversity of the Mediterranean diet a valid support to the environmental balance. The main aim of this researchs are to demonstrate how the improvement in food choices can contribute to increasing the environmental sustainability performance of the entire food chains analyzed in all phases of their life cycle. Barilla use for this objectives the Life Cycle Tools and verify the conditions for which it is possible to obtain a real trade-off between nutrition and environmental sustainability. The results of their mainly studies consist in the verification of the most recommended products in the diets for their nutritional indicators have also a lower impact on the environment. The results are shown alongside the traditional food pyramid; the food pyramid is built according to the ecological footprint of each of them. In terms of the life cycle, a factor that is still very critical and needs development is the estimation of the environmental impacts of the cooking phase.\nAlong the food chain there is a need to establish which are the key variables for the estimation of the same, how to build inventories, which impact categories to use and at the same time what are the nutritional differences between raw and cooked food and the differences in terms food safety. The purpose of this research consists precisely in filling this gap and at the same time providing suggestions on the variables to be used.

4D-Risk Assessment (PC)

MOPC13

The STREAMcom model - Linking time variable exposure to effects on individuals, populations and community in realistic landscapes

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The ecological risk assessment of chemicals aims to protect non-target populations and the communities they comprise while the underlying effect assessment is largely based on individual level toxicity information. Effects caused by the exposure of organisms to chemicals can however to a great extent depend on environmental scenarios as well on the states, behavior and interactions of organisms with consequences for individual life history, population responses and community dynamics. The quantitative contribution of the environmental scenario, including biotic interactions, to the chemical effect in a multiple stressor situation is however unclear. For analyses, we use the STREAMcom model, which integrates spatial explicit habitat information in form of raster maps, temporal information on abiotic factors such as temperature and chemical exposure, functional trait data bases, dynamic energy budget models and process based effect models to simulate macroinvertebrate assemblage and fish dynamics. In model applications, we explore to what extent the ecological scenario will affect the adverse outcome of chemical exposure.

MOPC14

Accounting for environmental complexity in pesticide risk assessment by the use of integrated models

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Environmental risk assessment (ERA) of pesticides is under continuous discussion. This asks for refined and more realistic ERA approaches for pesticides, which account for the variability of environmental factors and differences in landscape structures. On the other hand, authorities are already challenged by the effort to evaluate complex ERA dossiers, especially those from higher tier refinements. This contribution discusses the hypothesis that the development and the use of integrated mechanistic models for exposure and effect dynamics within a landscape context can account for parts of the environmental variability, increase the real-world relevance of ERA for pesticides and at the same time provides options to optimise risk mitigation and management. It is postulated that this can be achieved without further increasing the complexity of the ERA process when trusted modelling approaches would be available. A simple application example for integrated exposure and effect modelling in a landscape context showed that following the application of cypermethrin, about 10% of the ditches were within a factor of 10 from the onset of effects only, whereas about 40% of the ditches had no exposure or no risk. This appears as typical example for an agricultural landscape, and indicates how by an integration of fate and effect modelling the variability of environmental factors, in this example temperature and variation of hydrology in a small catchment, can be explicitly considered for exposure assessment and connected to state of the art effects analyses by TKTD models. The results raise the question of appropriate protection goals for such landscape-scale risk assessment results. In general, an impact assessment step where spatially and temporally explicit calculations of integrated risk measures in a landscape context could be added to the current generic risk assessment as a reasonable extension of the current RA paradigm. Such impact assessment would require the systematic collection of information on landscape properties, pesticide use, crop coverage, in combination with the further development of integrated exposure and effect modelling approaches that can be applied at large scales.

MOPC15

Aquatic risk assessment at landscape scale - conceptual development and an application example

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Risk assessment at landscape scales is currently discussed in Europe as a serious option to improve the realism and relevance of regulatory Environmental Risk Assessment (ERA) for plant protection products (PPP). Conceptually, geographical information can be used by spatially distributed models to predict the emission, exposure and environmental effects of the use of PPP at the region scale. This enables the assessment of local specific risks with a high level of realism. In practice, however, several challenges need to be solved before such landscape scale ERA can be used in a regulatory context. For example, there is a need for a proper framework for linking protection goals to landscape scale assessment endpoints, including associated spatial and temporal scales. Also, the multitude of possible combinations and complexity of different exposure and effect assessment and modelling approaches needs to be organised and selection criteria identified. Other issues to be addressed are the identification of the relevant environmental parameters, uncertainty propagation and how to present the assessment endpoints and the definition of decision making criteria for the risk evaluation. Our contribution will show preliminary results of a new project that aims to provide a possible framework for organising the challenges given above and develop practical modelling tools for aquatic ERA at landscape scales. First a systematic and comprehensive characterisation of possible modelling approaches will be presented. Secondly, an example will illustrate how landscape scale modelling results can be translated into predicted risk for the survival of aquatic macroinvertebrates.

MOPC16

Outcomes from an ECETOC Task Force on Geospatial approaches to increasing the ecological relevance of chemical risk assessments.

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For several decades, the prospective risk assessment of chemicals has followed a generic approach of comparing estimated exposures to toxic thresholds designed to be protective of all species (i.e., assessing exposure to the most sensitive species assumed to be located anywhere the chemical may occur in the environment). This approach does not recognise geographic patterns of species distributions or acknowledge that particularly sensitive species may not occupy potentially exposed habitats. Therefore, risk assessments could be overly conservative and restrictive for some uses of chemicals. Approaches for making spatially explicit assessments of chemical exposure are relatively advanced but this is not the case for mapping and assessing ecological data. However, geo-referenced ecological data appear to be increasingly available at spatial resolutions applicable to chemical risk assessment, potentially facilitating enhanced environmental relevance of such risk assessments. In 2017 a Task Force was initiated by European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) to assess the capability of making chemical risk assessments using available geospatially referenced chemical exposure, ecological receptor and ecosystem services data. Two case studies were developed to illustrate the potential to assess geo-referenced risks to ecological receptors in fresh water and terrestrial environments exposed to i) a chemical used in consumer cleaning products discharged via municipal WWTPs across the EU and ii) a range of representative active ingredients used in plant protection products on selected crops in Germany. After initially compiling a catalogue of available geo-referenced ecological data for Europe, geo-referenced exposure concentrations were derived by combining accessible chemical use and fate data with conventional exposure models. However, use of many ecological data sets over a pan-European range proved problematic due to data access issues, limited geographic coverage and unreliable quality. Nevertheless, several suitable ecological data sets were accessed after making specific requests to various organisations within national authorities and these were integrated with the exposure maps. The results of these case studies give an indication of the potential value of making geo-referenced chemical risk assessments as well as the limitations to current capability.

MOPC17

briskaR: A spatio-temporal exposure-hazard model for assessing environment risk of Bt-maize on Non-Target Lepidoptera

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Field cultivation of Genetically Modified Bt-plants has a potential environmental risk toward non-target organisms (NTOs) such as butterflies larvae through the consumption of Bt-maize pollen. Bt-maize produces a toxic protein, Cry, acting as an insecticid on pest such as the European Corn Borer (*Ostrinia nubilalis*) or the Mediterranean Corn Borer (*Sesamia nonagrioides).* While the Bt maize primarily targets pests that are detrimental to the crop, the Bt toxin is also active against some NTOs that might be of conservative interest. As the Bt toxin is expressed in pollen which is dispersed by wind, exposure to Bt toxin is likely to occur outside maize fields over large distances and can thus reach habitats of NTOs. In order to assess the current environmental risk of Bt maize at landscape scales and how management measures might mitigate the risk in a given agro-ecosystem, modelling tools are needed. Over the last ten years, several models have been proposed for assessing the risk of Bt-maize on non-target Lepidoptera. Whereas there is a better understanding of factors affecting mortality of non-target populations, the management of GM crops at the landscape level for protecting NTOs is still at an early stage of development. High spatial and temporal variability of ecosystems challenges the extrapolation of ecological effects of toxicants from one-dimensional tests. As a consequence, there is a controversy on the actual risk for sensitive species as well as on the management measures (e.g., isolation distances) that should be implemented. The generic R-package *briskaR* implements a spatially-explicit exposure-hazard model for environmental risk assessment, and can be used for assessing the impact of Bt-maize on non-target organisms. The model considers the dynamics of pollen dispersal of each individual maize field as well as the dynamics of each Lepidoptera group of larvae in order to infer the larvae exposure to Bt toxin. Then, using the exposure model, a toxicokinetic-toxicodynamic model accounts for the impact of the dynamics of toxin ingestion on lethal and sublethal effects at individual larvae level. The present spatio-temporal modelling application on Bt-maize impact on Lepidoptera is dedicated to be a support for environmental risk assessors and managers in running quantitative risk assessment. It also allows to test management strategies within an explicit landscape approach.

The Overlooked Hazard of Small Creeks - Elucidating the Input and Fate of Organic (Micro-)pollutants in Streams (PC)

MOPC19

Simulation of Diclofenac concentrations in small German rivers with GREAT-ER

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Simulation models can be used to estimate predicted environmental concentrations (PEC) based on chemical and physical properties and consumption data. The Geography-referenced Regional Exposure Assessment Tool for European Rivers (GREAT-ER) constitutes a model tool for exposure and risk assessment of chemicals in surface waters. The main objective of this study is to show that the model can be used to support risk management of surface waters not only in large rivers with high flow values, but also in small creeks receiving wastewater from treatment plants with unsatisfactory dilution ratios. The study area contains the German part oft the catchment of the river Vecht, whichbecomes a tributary to the dutch river Ijssel later on. This basin covers an area of only 1800 km², but covers with around 400,000 inhabitants the relatively large amount of 22 wastewater treatment plants (WWTPs) and six hospitals. The Vecht river itself is a medium sized cross-border river with many small tributaries of mean discharge values below 2 m³/s. Model simulations intend to show the impact of emissions of pharmaceuticals into small creeks with unsatisfactory dilution factors. In addition to the emissions of sewage treatment plants, emissions from hospitals in the catchment area will be taken into account. In this work, the focus is on the application of the model to Diclofenac. Since the drug is not completely removed during conventional sewage treatment, it is detected almost ubiquitously in European surfacewaters. Although Diclofenac is prone to photodegradation in surface waters, concentrations in surface waters may exceed the environmental quality standard (EQS). Thus, Diclofenac was added as priority substance under the EU Water Framework Directive, but monitoring efforts are often focusing on the large waterways neglecting smaller creeks and tributaries due to their small flow values. To represent the status quo of Diclofenac contamination in the whole Vecht watershed including small creeks, probabilistic simulations with 10,000 runs were performed. Simulations show that exceedance of the EQS is predicted to occur in a number of small creeks as well as in parts of the Vechte river itself. Predicted PEC/EQS exceedances are in a range also measured by monitoring campaigns in Europe. Evaluation of simulation results will be performed by comparison with measurements to be obtained in the Meduwa project later.

MOPC20

Combined biological and chemical assessment of natural estrogen input from agriculture in tributary waters of Lake Baldegg

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Estrogens in surface waters have negative effects on reproduction and development of aquatic organisms [1]. Agriculture may be a significant source for natural estrogens in surface waters [2]. Several studies in different countries have demonstrated that the application of manure from husbandry animals to soil causes the release of natural estrogens to surface waters [3, 4]. As it is still unclear for Switzerland, we aimed to determine the contribution of agricultural-derived estrogens to the total estrogen load in surface waters. Our study area is the catchment of Lake Baldegg, where the highest husbandry animal densities in Switzerland, meaning 2.5 livestock units per hectare, are situated. Consequently, large amounts of manure are produced and applied in this catchment, leading to potentially elevated concentrations of natural estrogens in surface waters. To verify this hypothesis, we took water samples of the small to medium sized tributary waters of Lake Baldegg. All sampling sites are free of wastewater treatment plant effluents to ensure that we measure only agricultural-derived estrogens. In the vegetation period 2018, we took water samples every 22nd day. Only in March 2018, 17ß-estradiol concentration was above limit of quantification in four tributary waters. Based on our findings in the vegetation period 2018, we are currently studying more systematically the natural estrogen input in the tributary waters of Lake Baldegg. In order to capture peaks of natural estrogen input in the tributary waters of Lake Baldegg, we are collecting daily time proportional composite water samples for 30 days. We will chemically analyse the concentration of estrone (E1), 17α-estradiol (E2α), 17β-estradiol (E2β), estriol (E3) and total phosphorus. Additionally, we will daily measure average discharge for every sampling site to calculate loads of natural estrogens emitted. Furthermore, we will weekly assess estrogenicity of the tributary waters with ERα-Calux bioassays. We aim to present results of the sampling campaign in which we monitor the estrogen input in the tributary waters of Lake Baldegg systematically for 30 days. References: 1. Nakamura, et al., Fish Physiol Biochem., 2003. 28(1): p. 113-117. 2. Johnson, et al., Sci Total Environ, 2006. 362(1): p. 166-178. 3. Kjær, et al., ES&T, 2007. 41(11): p. 3911-3917. 4. Dutta, et al., J. Environ. Qual., 2010. 39: p. 1688-1698.

MOPC21

Assessing the micropollutant burden in small agricultural streams using in vitro bioassays

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Micropollutants can enter small streams from a number of sources including point sources, such as wastewater effluent discharge, and diffuse sources, such as agricultural run-off. The current study aims to assess the chemical burden in small agricultural streams using cell-based bioassays in parallel with chemical analysis. Water samples were collected from over 40 sites in eleven German states from April to September 2018 with sampling triggered by rain events. The water samples were extracted with solid-phase extraction and run in *in vitro* bioassays indicative of activation of the aryl hydrocarbon receptor (AhR), binding to the peroxisome proliferator-activated receptor (PPARγ), activation of the estrogen receptor (ER) and oxidative stress response. While effects in the extraction and solvent blanks were very low, the majority of samples induced a response in the studied bioassays, with a wide dynamic range observed. Binding to PPARγ and activation of ER were the most responsive assays, with some of the samples exceeding preliminary effect-based trigger values for surface water for activation of AhR, activation of ER and oxidative stress response. To better understand which chemicals were contributing to the observed effect in the bioassays, the effects of some of the detected chemicals were fingerprinted in the bioassay test battery. Iceberg modelling using the bioanalytical equivalent concentration (BEQ) approach was applied to determine the fraction of the effect explained by detected chemicals. This study shows that small streams can have a considerable micropollutant burden, emphasising the need for reevaluating regulatory monitoring measures.

MOPC22

Impact of organic micropollutant dynamics on the water quality of a small river

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Rivers integrate water of their entire catchment and pose an important environmental compartment in which transport and transformation processes of anthropogenic chemicals take place. To trace changes in the water quality and to identify potential input sources of organic micropollutants into the river an integrated analytical and bioanalytical approach was applied. One stretch of the Ammer River, a tributary of the Neckar River close to Tübingen (South-western Germany), was sampled in July 2018 at two sites downstream of a wastewater treatment plant (WWTP) with large volume-solid phase extraction (LV-SPE) devices. Both LV-SPE devices integrated a total period of 24 hours divided into four sampling intervals. Additionally, grab samples of tributaries of the river between both sampling sites were collected. The water extracts, including extraction blanks, were measured by three *in-vitro* bioassays covering the environmentally relevant modes of action: activation of the aryl hydrocarbon receptor (AhR), estrogenicity (ER), and oxidative stress response (AREc32). In addition, mitochondrial toxicity was measured by the cellular oxygen consumption rate (OCR) assay using the Agilent Seahorse XF^{e}96 Analyzer. Furthermore, all samples were screened for 88 target analytes by LC-MS/MS. Iceberg modelling using the bioanalytical equivalent concentration approach was applied to estimate the contribution of the detected target analytes to the observed effects. The water extracts showed temporal and spatial variability in the assays AhR and AREc32 with most samples being active. The OCR assay showed initial uncoupling activity at the upstream sampling site just below the WWTP, which transitioned to marginal uncoupling at the same sampling site at later sampling periods and strong electron transport chain inhibition at the downstream site. The uncoupling effect coincided with estrogenic activity, which is a marker of the water being dominated by wastewater treatment plant effluents. In all other samples and in the tributaries the mitochondrial toxicity was dominated by inhibition of the electron transport chain. This study shows the dynamics of organic micropollutants in one stretch of a small river and demonstrates that the impact of anthropogenic chemicals on the water quality maybe highly variable. This work was supported by the Collaborative Research Centre 1253 CAMPOS (Project P1: Rivers), funded by the German Research Foundation (DFG, Grant Agreement SFB 1253/1 20147).

MOPC23

Coupling of water quality and toxicokinetic models to predict the internal concentrations of wastewater-derived chemicals in gammarids in small streams

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Water quality models for predicting environmental concentrations of target contaminants are well established within the environmental chemistry field, but the use of toxicokinetic (TK) modeling for estimating internal concentrations in target organisms remains underutilized in exposure science. A combination of both modeling techniques can be indispensable (e.g., water quality and TK), especially when information on the mechanistic linkages between contaminant exposure and effects is eventually desired. In this study, we employed a model that first predicted the time-varying concentrations of 36 wastewater-derived compounds downstream of wastewater treatment plants for a year. Then, we coupled the results to a toxicokinetic model that estimated the internal concentrations in field gammarids. The models were intended to predict transient conditions (non-steady state) which were then compared to the measured river and internal concentrations. The simulations yielded satisfactory results which demonstrate the utility of the coupled models in describing the temporal dynamics of contaminant exposure at sites previously determined to be heavily impacted by wastewater-derived chemicals. A similar work is currently being undertaken for high-flow conditions. In the future, the time-varying field simulation of exposure media and internal concentrations will be combined with higher-level effects modeling to finally link exposure and effect.

MOPC24

Hazardous chemical impacts of effluents from pharmaceutical industry on sediment quality in a small stream

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Surface waters often serve as primary recipients of wastewater effluents from urban agglomerations and industrial facilities. Due to their low base-flows, small, wadeable streams are especially vulnerable to increased anthropogenic inputs. In this work, we investigated distribution of hazardous chemical contaminants in a small stream, receiving effluents from pharmaceutical industry and bakers's yeast production. Samples of surface sediments were collected at 20 locations along the longitudinal profile of the Gorjak creek, located near the city of Zagreb, Croatia. The aim of the study was to assess the sediment quality, in particular to reveal the signatures of the contamination related to industrial activities. The analytical approach comprised comprehensive analysis of both organic and inorganic constituents, using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for a wide-spectrum analysis of organic contaminants and inductively coupled plasma mass spectrometry (ICP/MS) for multi-elemental analysis, covering 25 metals and metaloids. The results revealed a strong impact of industrial inputs on the quality of the examined sediments. The impact of baker's yeast production was reflected primarily in significantly enhanced levels of organic matter. However, the major concern is related to high levels of some toxic contaminants such as polycyclic aromatic hydrocarbons, surfactant-derived compounds, and a suite of pharmaceutical compounds (macrolide antibiotics, zolpidem, torsemide, chlorthalidone, terbinafine and warfarin) as well as some toxic metals (Ni, Hg, Cu and Pb). The longitudinal distribution of all contaminants in this predominately lentic system rapidly decreased with the distance from the wastewater discharge point, indicating their fast deposition near the source. Further longitudinal transport was probably associated with increased stream water discharges following rain events. It was noticed that the geochemical fractionation and the mobility resulting from the transport of contaminated sediments was different for different contaminants. Nevertheless, several kilometers downstream, the concentrations of the majority of critical contaminants returned to the levels close to the background values.

Bees, Bugs and Beneficials in Environmental Risk Assessment and Testing (PC)

TUPC01

Heterogeneous residues change pesticide-induced behavioural responses in predatory mites

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For a pesticide to be approved for use, the lethal and sublethal effects on non-target arthropods (NTAs) must be studied. Reproductive effects are routinely explored, but behavioural changes are less well understood. Such changes have been reported in NTAs exposed to some pesticides, with avoidance behaviour, where individuals display signs of irritation or repellence when exposed to residues of a pesticide, being of particular interest. Moreover, NTA responses to heterogeneous pesticide coverage also requires further investigation. We aimed to develop an efficient method to quantify changes in individuals' movement behaviour and thereby identify avoidance behaviour in relation to pesticide exposure in the predatory mite *Typhlodromus pyri*, a model species and natural predator found in fruit orchards globally. Using video analysis, we exposed individual adult mites to 3 insecticidal active ingredients (acetamiprid, deltamethrin, dimethoate), each at 3 concentrations, and evaluated mite movement behaviours when exposed to these in choice and no-choice test arenas. Residues were at the same level per unit of treated area in both studies. We report the varied effects on a range of movement behaviours in *T. pyri*, including distance walked, time moving, velocity and meandering behaviour. We also discuss avoidance behaviour and compare responses in choice and no-choice exposure scenarios. In no-choice arenas, we found that deltamethrin residues of 0.002 g Ha-1 reduced distances walked by mites by up to 87% compared to the control, and that 54% of individuals exhibited repellence through becoming trapped in the test arena boundary compared to 0% in the control. However, these effects were not observed in mites exposed to the same residue rates in choice arenas, with only 17% of mites becoming trapped at the 0.02 g Ha-1 rate in choice arenas. In choice arenas, mites spent less time on deltamethrin residues than on untreated surfaces. We observed no clear effect of acetamiprid on behaviour. When in choice arenas, mites preferred to avoid dimethoate, thereby reducing contact with residues, though no clear response was observed in no-choice arenas. Our studies highlight the need for further understanding of how NTAs respond behaviourally at a micro scale to heterogeneous exposures of pesticides. Our findings can be used to improve realism in pesticide risk assessment and complements the existing knowledge of sublethal pesticide effects in NTAs.

TUPC02
Mayflies in Ecotoxicity Testing: Methodological Needs and Knowledge Gaps Identified in a Virtual Workshop
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Among the sensitive EPT (Ephemeroptera, Plecoptera, Trichoptera) group of insects, mayflies (Ephemeroptera) have been applied most successfully in water and sediment toxicity testing. For example, the recent development of a culture-based, life-cycle test with *Neocloeon triangulifer* and the long-standing sediment test with *Hexagenia* in North America, and the routine application of field-collected *Cloeon dipterum* in European testing reflect commensurate advancements in understanding the biology of these organisms and their behaviour under lab holding and testing conditions. With growing interest in expanding the arsenal of mayfly species used in toxicity testing, a virtual workshop was held in September 2018 to identify and discuss knowledge gaps constraining advancements in the use of new mayfly species in culture-based or field-collected protocols for water and sediment toxicity testing. Participants from industry, government, and academic sectors, representing both Europe and North America, took part in the workshop. Discussions were guided by several questions: 1) Should the emphasis be placed on further development of lab-cultured species or on a more standardized use of field-collected species? 2) What criteria should be used to select suitable test species (e.g., relative sensitivity to chemicals and test conditions, ecological traits, culturability)? 3) Should regulatory requirements play a role in determining species selection? 4) In tests emphasizing field-collected species, how can holding, feeding, and testing conditions be improved to maximize organism health and successful use in testing? In this poster presentation, we summarize the outputs of the workshop using, where appropriate, a data-driven approach based on species sensitivity distributions comparing the relative sensitivity of mayflies to various chemical classes based on ecological traits classification. *Disclaimer: This presentation does not necessarily reflect the views or the policies of the U.S. Environmental Protection Agency.*

TUPC03
Which is the most suitable native bee species from the Neotropical region to be proposed as model-organism for exposure to pesticides during the larval phase?
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Brazil has the greatest diversity of native stingless bees of the world and makes intensive use of pesticides. Studies on larval phase focus on *Apis mellifera*, since for this species the rearing method is already standardized by the OECD*. An *in vitro* larval rearing method has already been described for the Brazilian native stingless bee species *Melipona scutellaris*, for application in studies of exposure to pesticides during larval phase. Our study aimed to propose an *in vitro* larval rearing method for other stingless bee (*Scaptotrigona postica*), and to compare the results to those obtained from *M. scutellaris*. The acrylic plates with 100 brood cells were placed in glass Petri dishes containing distilled water. Larval food was collected from brood combs, each artificial cell receiving 25μL of larval food and, afterwards, 24-hour-old larvae were placed in the food. Then, the plates were kept in an incubator at 30ºC and 75% of relative humidity. After 72h and 120h of the larval transference, the humidity within the Petri dishes was reduced, respectively, to 85% (adding KCl) and to 75% (adding NaCl). This technique was carried out three times (total 900 larvae), evaluating (A) developmental standards, (B) emergence and mortality rates, and (C) morphometry of newly emerged workers (from *in vitro* and *in vivo*). T test for independent compared the means of the morphometry from both experiments. Moreover, we applied the method for determining (D) the average lethal concentration (LC_{50}) of the insecticide dimethoate. We also compared our results from the B and D parameters to those already stablished for *A. mellifera*. Regarding the emergence and mortality rates, *S. postica* produced above-expected results, comparing to those of *A. mellifera*, and even those of *M. scutellaris*. The LC_{50} of dimethoate indicated that *S. postica* is, approximately, 3 times more sensitive than *M. scutellaris* and, about 1047 times more than *A. mellifera*. The T test indicated similarity between *in vivo* and *in vitro* experiments for morphometric parameters in *S. postica*, as was also observed in *M. scutellaris*. Considering *S. postica* and *M. scutellaris* species, our results lead us to point out the first one as the most suitable native species to be proposed as model-organism. We presented the first comparative approach of responses to *in vitro* larval rearing methods between two native bee species from Neotropical region, for use in risk assessment.

TUPC04
Guttation as an exposure route in the risk assessment for plant protection products - Review of the available data
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While the risk assessment scheme for bees proposed by EFSA (2013) is not yet in force, additional studies and endpoints, as well as the assessment of additional exposure routes, are already required for the registration of Plant Protection Products (PPP). One of these exposure routes is via the foraging on guttation droplets potentially contaminated with PPP residues. A standard procedure to assess the risk in the regulatory context of PPP is the comparison of toxicity and exposure. While the toxicity is inherent to a certain substance and can be measured in standardized laboratory tests, the exposure part in connection with guttation is more complex. Three elements need to be considered in order to assess exposure via guttation to foraging honey bees: 1. The amount of residues in guttation water after PPP application. 2. The occurrence of guttation on a certain plant species. 3. The extent to which honey bees are actually foraging on guttation droplets. The proposed assessment scheme (EFSA 2013) for risk the from guttation water is highly conservative, which often leads to the need for a higher tier risk assessment. While it is possible to conduct higher tier studies e.g. in a residue trial or field effect study for the specific substance, the data that is already available should also be considered, especially for the more generic, i.e. not substance specific, part (points 2 and 3). In this review we evaluated field studies on guttation, which were conducted by the industry for registration purposes between 2010 and 2017. Aim was to find a realistic estimate (90th percentile) for the occurrence of guttation in a certain crop species as well as an estimate for the number of honey bees foraging on guttation water (90th percentile) on a certain crop. Results show that guttation was frequently observed for numerous crops. The number of honey bees actually foraging on guttation droplets was low for all crops. Data which was available for two seasons (autumn and spring) show that there was a tendency that more honey bees were observed taking up guttation fluid in spring. As the uptake of guttation fluid by honey bees foraging for water seems to be a rare phenomenon, it could be worthwhile to re-assess the risk by the exposusre to guttation water for honey bees in order to come to a more realistic and not overly conservative risk assessment.

TUPC05
Effect of agricultural landscape structure on response of the ground beetle Poecilus cupreus to Proteus 110 OD insecticide
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Intensification of agriculture and the widespread use of pesticides during the last few decades have led to significant reduction of the abundance of non-target arthropods (NTA). One of the main reasons of this is the widespread insecticides use, focusing on eradication of pest species without paying attention to NTA. In addition, the growing demand for agricultural products leads to changes in agricultural landscape, resulting in disappearance of non-cultivated elements (field margins, hedgerows, meadows). In this study we investigate the effect of agricultural landscape structure on response of the ground beetle *Poecilus cupreus* to Proteus 110 OD (active ingredients: Thiacloprid and Deltamethrin), the insecticide commonly used in the study area. Beetles were collected from two types of agricultural landscapes in central Poland, dominated by small- or large-scale farming (S & L, respectively). Within each landscape type 9 sites were established. In S landscape there were 3 reference sites (meadows) with 0 oilseed rape coverage (ORC) (S0), 3 sites with small ORC (10-14%; SS), and 3 sites with medium ORC (20-52%; SM). In L landscape there were also 3 reference sites with 0 ORC (L0), 3 sites with medium ORC (28-33%; LM), and 3 sites with large ORC (80-98%; LL). Beetles were exposed individually to a single topical application of 1 µl of the insecticide solution or to control treatment (acetone) applied on scutellum with a Hamilton syringe with repeater. The commercial formulation Proteus 110 OD was dissolved in acetone to obtain the concentration equivalent to 0.2 recommended concentration for field use. Statistical test comparing all habitats together revealed statistically significant differences in beetles survival ($p< 0.001$). Further pairwise comparisons showed that meadows S0 ($LT50=7{,}31 \pm 4{,}42$ SE) and L0 ($LT50=10{,}27 \pm 1{,}97$ SE) did not differ from each other ($p=0.649$), and the only groups differing from these reference habitats were the two agricultural habitats from the L landscape: beetles from LM ($23{,}26 \pm 1{,}64$ SE) and LL ($21{,}25 \pm 3{,}41$ SE) habitats survived significantly better than any other group. Our results indicate, that *P. cupreus* beetles from habitats with medium and high ORC have elevated resistance to the commonly used insecticide, but only if they live in large-scale agricultural landscape. This study was supported by National Science Centre, Poland (2016/23/N/NZ8/01679) and Jagiellonian University in Kraków, Poland (DS/WBiNoZ/INoS/759/2018).

TUPC06

The importance of synergy for risk assessment: pesticides, nutrition, and behaviour
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Animals are exposed to multiple stressors simultaneously, such as pesticides (including chemical mixtures of multiple active ingredients), nutrition deficiencies, and diseases. Combined exposure to multiple stressors can cause interactive effects, amplifying the risk if the two agents (i.e. two pesticides) interact synergistically altering animal survival and behaviour. However, the Regulatory Risk Assessment (RA) generally evaluates the risks posed by only one agent at the time (i.e. one pesticide), and mainly addresses effects on animal survival. Risk assessors have identified the RA of chemical mixtures as one of its current priority tasks, but synergistic effects on animal behaviour, as well as synergies caused by non-pesticide stressors, are not yet implemented in the RA. The lack of appropriate scientific studies and methodologies about these key topics partially explains the respective lack of advances in RA. However, the difference between the complexity of real-world situations (i.e. exposure to multiple stressors affecting survival and behaviour) and the simplified RA scenario can lead to uncertainties in RA conclusions. Thus, we developed methodologies to assess synergistic effects of key animal stressors on bee survival and behaviour. Our work was based on the LD_{50} standard test, which is already commonly used in RA, to facilitate implementation and comparisons. Honey bees were used as RA animal model. Our work demonstrated that animal health can be synergistically impaired by the combination of two major common stressors, pesticides and poor nutrition, at field-realistic exposures. We also demonstrated that combined chemical stresses synergistically impaired animal behaviour and survival.

Fish Model Species in Human and Environmental Toxicology (PC)

TUPC07
Effects of pH on toxicity and bioaccumulation of ionisable substances using the fish embryo test (FET) with Danio rerio
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The fish embryo test (FET) with *Danio rerio* is a well-established and standardised (OECD Guideline 236) *in vivo* toxicity test that is commonly used to evaluate potential effects on early development of fish. Thus, it is a helpful tool for risk assessment in aquatic environments. However, until now, neither pH nor the particular properties of ionisable organic compounds (IOC) have sufficiently been considered in risk assessment. Characteristics of IOC vary, depending on their presence either as ions or as neutral species, in particular in respect to the uptake into organisms. Due to their electrical charge, ions pass poorly through biological membranes, whereas neutral species permeate more easily through membranes and are, thus, potentially of higher toxicity. The pH is one major factor influencing the proportion of dissociated and non-dissociated ions. Shifts in ambient pH cause alterations of the ionic proportions and thus, are key to IOC toxicity. Although many IOC are partly or completely ionised under environmental relevant conditions and already slight variances of pH can cause considerable changes in toxicity, little attention has been paid to pH and its consequences in toxicity testing. Therefore, the project PHION focusses on pH-induced effects of IOC in *Danio rerio* embryos. Apart from differing toxicities, also pH-dependent bioaccumulation is investigated based on the lethal body burden (LBB) concept which assumes that there is no toxicity without previous bioaccumulation. The goal is to evaluate to which extent pH is influencing IOC uptake, potential bioaccumulation and toxicity on the basis of 30 representative substances to eventually derive a generally valid prediction on the toxicity of compounds previously not being tested at different pH. The FET with *Danio rerio* will be complimented with acute and chronic tests with *Daphnia magna* and *Lemna gibba* to compare the potential pH-dependent effects of IOC at three different trophic levels. Data presented refer to *Danio rerio* FET results for the first subset of ionisable pharmaceuticals.

TUPC08
The Baltic populations of Atlantic salmon (Salmo salar) at risk? A multi-biomarker study as contribution to the understanding of a pressing health issue
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The Baltic Sea is highly influenced by its nine neighbouring countries. River discharges from highly populated and agricultural areas, pollution from marine traffic and other anthropogenic activities as well as multiple other biotic and abiotic factors challenge the marine organisms inhabiting the Baltic Sea. Atlantic salmon (*Salmo salar*), one of the top predators and a migratory keystone species has been facing severe health issues for half a decade. Within the last 5 years, symptoms such as skin haemorrhage, traumatic injuries and ulcers were reported from different rivers mainly from Sweden and northern Finland. These primary symptoms precede a secondary fungal infection (mycosis), lethargy and ultimately high mortality rates. Even though research has been conducted on the Baltic salmon populations, the focus has been towards yolk-sac fry mortality (commonly known as M74 syndrome), which is caused by thiamine deficiency. Recent investigations into the current health issues of returning salmon have not yet elucidated the main causative factors. In the summer of 2018, returning salmon were caught from four rivers along the Swedish east coast from Torne river in the north to river Mörrumsån in the south. Moreover, a sampling site on the west coast (river Lagan) was included. Different tissues were collected for assessment of (eco)toxicological parameters, which will be evaluated by using common, pollution-driven biomarkers. Disease prevalence will be assessed, using different criteria (e.g. histopathology). Furthermore, levels of vitamins (including thiamine) and antioxidants are going to be analysed. The oxidative stress-related hepatic enzyme activities of catalase (CAT), glutathione reductase (GR) and glutathione *S*-transferases (GST) were analysed. In plasma, ions (i.e. K^+, Na^+, Ca^{2+}, Cl^-) were determined Immunological assays, i.e. lysozyme and complement system activities in plasma and other biomarkers (e.g. protein oxidation, AChE and EROD activity) will also be included in the final assessment and presented at the SETAC conference. Preliminary results on oxidative stress show slight differences between mycotic and non-mycotic fish, but no clear regional patterns were observed. Initial data analysis on plasma ions shows reduced levels of Na^+ and Cl^- in fish with mycosis, but no conclusive regional pattern. With this comprehensive study, we hope to shed more light on the health status of Baltic salmon.

TUPC09
Evaluation of the acute toxicity, uptake and biotransformation potential of citalopram in zebrafish (Danio rerio) embryos at three environmentally relevant pHs
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The contamination of the aquatic environment has raised concerns in the scientific community and regulatory authorities. Given the large number of xenobiotics, for most of them there is a striking deficit in the literature concerning their adverse effects on aquatic organisms. Citalopram (CTR) is a worldwide highly consumed antidepressant which has demonstrated incomplete removal by conventional wastewater treatment, hence resulting in the contamination of aquatic ecosystems. Consequently, it is urgent to evaluate its potentially toxic effects on aquatic organisms. Zebrafish (*Danio rerio*) has emerged as a powerful model organism to study various aspects of developmental and cell biology as well as physiology, while it provides a promising alternative model for acute toxicological studies. The objectives of this study were (1) to assess to what extent CTR induces toxicity to zebrafish embryos. In addition (2), we evaluated the uptake and biotransformation processes of CTR by zebrafish and examined whether biotransformation data could be used in a complementary way to the concentration of the parent compound to interpret the induced toxicity. The final goal was to evaluate to which extent the pH is influencing CTR's uptake, potential bioaccumulation and biotransformation, as well as toxicity. More specifically, the zebrafish embryo toxicity assay was used to calculate the LC_{50} of CTR, as well as to evaluate potential sub-lethal endpoints (e.g. the hatching rate). Exposure experiments were conducted at three different pH values (6, 8 and 9), to assess potential pH-dependent differences in an environmental relevant pH range. Concerning the toxicokinetic part of the study, exposure water samples and zebrafish extracts were analysed by RPLC and HILIC methods, in both positive and negative ionization mode, to cover the widest possible range of polarities, using LC-QTOF-HR-MS/MS. Detection and identification of tentative CTR biotransformation products were performed through in-house developed suspect and non-target screening workflows. Finally, the biotransformation pathway of CTR in zebrafish embryos was proposed.

TUPC10
Organochlorine pesticides in Tigerfish (Hydrocynus vittatus) in Phongolo, South Africa
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Persistent organic pollutants like organochlorine pesticides (OCPs) are common environmental pollutants. Although these compounds have been banned because of various detrimental effects, including cancer, they are still found in the environment due to their persistence, and widespread and large volume use. Dichlorodiphenyltrichloroethane (DDT) is a banned OCP in most parts of the world, but is permitted for use to combat the malaria vector mosquito. Phongolo is one of the areas where it is still used. Due to bioaccumulation it is expected that top predators would be especially affected by these compounds. For this reason concentrations of OCPs (DDT and metabolites, chlordane, hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), and aldrin, dieldrin and endrin that are collectively termed cyclodiens) were quantified in the top predator, Tigerfish (*Hydrocynus vittatus*). Muscle tissue was extracted using automated soxhlet and cleaned with Florosil solid phase extraction. The target compounds were quantified using gas chromatography electron capture detector (GC ECD). Concentrations of ΣDDTs were 11.1 ng/g wet mass (wm), with *p,p*-DDE making up the greatest proportion. ΣChlordanes were low (0.13 ng/g wm), and only trans-chlordane and trans-nonachlor having detectable concentrations of the eight chlordanes analysed. ΣHCH (four isomers) were low, 0.25 ng/g wm. Concentrations of HCB and cyclodienes were below the detection limit. Concentrations of DDTs and HCHs in this system were lower than those recorded in Tigerfish from rivers in and around the Kruger National Park, South Africa. Fish from the iSimangaliso Wetland Park, south of the current study, recorded similar to slightly higher concentrations to those reported here. Concentrations of DDT are a possibility for concern because of the various health implications and their bioaccumulation potential, indicating the food web is exposed to these compounds, and there is a great potential for humans to be affected. Monitoring of this system, and the health of the fish should be assessed in further studies.

TUPC11

METABOLOMIC ANALYSIS OF GONADS AND LIVER OF FISH EXPOSED TO EFFLUENT OF PULP AND PAPER MILLS.

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Effluents from pulp and paper mills are internationally recognized as containing toxic substances with properties to alter the reproductive capacity of fish. The objective of this study was to evaluate the metabolic modifications (using metabolomics tools) of fish gonads and liver (*Danio rerio*) exposed to effluent from the pulp and paper industry, as well as the embryonic development of embryos generated by the respective fish. The effluent was diluted by a factor of 1/25. The fish (10 males and females) were exposed to the effluent, and males and females were kept separated for 7 days at 28 ºC and a light / dark cycle of 12/12 h in 3.5 L glasses. After pre-exposure period, males and females were mated (1 couple per Becker containing a net at the bottom) and monitored for 6 days with daily water/pollutant renewal and fed twice a day. The eggs were collected and placed on plates containing the test agent, kept protected from light. The fish were sacrificed for liver removal and gonads, from which a homogenate was prepared. Then, extraction was performed with chloroform/methanol/water (3:2:1). The extract obtained was prepared for the analysis by gas chromatography (GC/TOF-MS). For the exposed fish, there was a significant reduction in egg production, 65%. A high percentage of the eggs presented dark staining, which is not feasible. Chromatography results revealed significant changes in the amino acid, sterol and fatty acid profile in both tissues, liver and gonads. The results showed a strong impact on the metabolism, egg production and embryo development for the studied fish, which point to the alteration of their reproductive capacity.

TUPC12

Histopathological changes in gills and livers of shorthorn sculpin (Myoxocephalus scorpius) exposed to lead

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Mining operations worldwide have caused significant heavy metals contamination of the local environment, including marine ecosystems. Sculpins have been used as fish species for environmental monitoring of heavy metal contamination such as lead and zinc in the mining areas in Greenland. However, the effects of heavy metals have not been validated in laboratory experiments. In this study, shorthorn sculpin (*Myoxocephalus scorpius*) were exposed to lead (Pb) in a controlled laboratory experiment. After acclimatization for two weeks, fish were placed in 4 tanks (15 fish/ tank) – 2 exposure tanks (200 μg/L Pb) and 2 control tanks. After 28 days of exposure, the fish were dissected and samples of gills and livers were collected. The samples were fixed in 10% buffered formalin. Samples were processed using standard protocols for histology, embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin (H&E). Metal analyses were done using ICP-MS. Pb was significantly higher in the liver of exposed sculpins (0.45±0.03; mean±SEM) than in the control fish (0.13±0.03; mean±SEM) (*p< 0.01*). The histological examination showed a number of lesions in gills (telangiectasia and lamellar fusion) and livers (megalocytic hepatosis). Relationships between heavy metals residues and quantitative histopathology of gills and livers will be presented and discussed. Overall, the results suggest that Pb exposure could alter structure of gill and liver of the sculpins. This will help to interpret results of sculpin studies from mining areas.

Fate and Effects of Metals: Advances in Metals Risk Assessment and Regulatory Guidance (PC)

TUPC13

Metal mixture toxicity on freshwater algal communities: the something-from-nothing effect

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Metal contaminations are still a likely reason for EU freshwater systems not to reach a “good ecological status”, as defined by the European Union Water Framework Directive. Risk assessment of contaminated waterbodies is largely focused on a metal-by-metal basis, while mixed contaminations are the rule rather than the exception. This is definitely the case for toxic trace metals, such as copper, nickel and zinc, which often occur close to their environmental limits. Mixture effects of these metals have been observed in individual species, even when each individual metal shows no effect, the metals were toxic when dosed together; this is called the something-from-nothing effect. In ecological communities, we hypothesise this effect can be even more pronounced due to competition effects, but experimental data on this subject is minimal. Here, we tested metal mixture toxicity on 8 freshwater algal species individually and in some selected combinations. The cell biomass of the species varies between 0.5 and 40 x10-5 ug/cell, growth rate between 1 and 1.7 day-1 and carrying capacity between 50 and 100 mg dw./L. Mechanistic modelling based on nutrient (phosphorus) uptake and above mentioned specific species properties was explored to help interpret the results. The idea being that the faster growing species will dominate under control conditions. While under metal mixture stress, the least sensitive species, i.e. the species with the highest toxic threshold to the individual metals and/or with the least pronounced something-from-nothing effect, will outcompete the other species. Experimental and modelling work is still in progress, but will be presented at the SETAC conference.

TUPC14

Testing performance of biotic ligand models for Cu, Ni and Zn in Northern European water chemistries

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The influence of water chemistry on the bioavailability and toxicity of metals is well acknowledged in the scientific literature. Accordingly, the EU has recently directed that the annual average Environmental Quality Standards for nickel and lead in surface freshwaters be set as bioavailable, rather than total dissolved concentrations. Also zinc, copper and manganese have bioavailable regulatory limits in use in some EU countries. For these metals, Biotic Ligand Models (BLMs) have been developed that predict their bioavailability under different physico-chemical conditions of the water. From the BLMs, practical and user-friendly tools have been derived for calculating the bioavailable metal concentrations using only few water quality parameters. Regulatory jurisdictions need to familiarize themselves with the models and find a framework to assess risks of metals in surface waters. Another challenge posed by the existing models is that they are not validated for all possible water chemistry conditions present in the EU. This is particularly evident for the Fennoscandinavian waters. The reason for lacking validation is that the standard test species used for the BLMs do not tolerate extreme conditions like highly acidic and very soft waters. This project aimed to fill this gap and address the challenge for the regulators in the Nordic countries. The project has produced chronic toxicity data using algae (*Pseudokirchneriella subcapitata*), daphnid (*Daphnia longispina*) and snail (*Lymnaea stagnalis*) conditioned or being indigenous for the low pH and low hardness conditions that can be observed in Nordic waters. The NOEC and/or EC10 values were obtained for copper, nickel and zinc. The full BLM models behind the Bio-met user-friendly tool estimated the EC10 values in the measured test water chemistries and the results were compared to the empirical endpoints. Eight surface water samples from Finland and Norway were tested and 28 modelled vs. observed pairs were produced. In general, for the daphnid and snail comparisons, the estimates stayed within a two-fold difference which is considered acceptable. In contrast, for the algae, all the observed EC10s were clearly below the modelled estimates. The results give a chance to evaluate the performance of BLM models below or close to the validation conditions and offer new data for further improvement of BLMs.

TUPC15

Critical assessment of methods for organic carbon analysis: significance for predictions of trace metal speciation and bioavailability in surface waters

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The development of robust approaches and criteria for protection of ecosystem functioning and ecosystem services is a challenging task that requires consideration of multiple abiotic and biotic stressors, the nature and relative magnitude of which vary across locations. Consequently, a single absolute threshold value for a given pollutant might be protective in one scenario and underprotective in another. Fortunately, legislators are increasingly recognizing the importance of contaminant speciation and bioavailability in environmental risk assessment. Under the European Water Framework Directive, the Environmental Quality Standards of some trace metals are already set as bioavailable thresholds e.g. zinc and lead. Such thresholds are typically derived from empirical correlations between the free metal ion concentration in the water and lethal accumulation and/or effect levels in biota as determined under laboratory conditions. Typically equilibrium-based metal speciation codes are used to compute free metal ion concentrations for the given water conditions. A key input parameter in this regard is the Organic Carbon (OC) content. OC forms complexes with a wide range of trace metal ions, the thermodynamic and kinetic features of which are dependent on the metal-to-binding site ratio. Thus, the predictive capability of modelling strategies may be affected by the accuracy of the OC data. During a large scale field monitoring campaign to assess links between metal speciation, bioavailability and potential toxicity, we encountered a discrepancy in measurements between the Dissolved- and Total Organic Carbon contents (DOC/TOC) when using standard protocols. Specifically, we observed the theoretically impossible outcome that DOC was higher than TOC, which led us to dig deeper into the methodology for OC measurements. In our presentation we will show several protocols for measuring both DOC and TOC, and the consequent impacts on the (i) metal speciation computed by various chemical speciation codes, e.g. Visual MINTEQ, WHAM,… and (ii) toxicological risk assessment predicted by e.g. PNEC-PRO, BIO-MET…. The outcomes of our study provide greater insight into the validity of OC measurements and will contribute to the development of more robust approaches for linking metal speciation to bioavailability under field conditions.

TUPC16

Toxicity assessment of nickel to Daphnia magna in Japanese natural waters

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To predict toxicity of nickel to aquatic organisms in natural water, nickel biotic ligand models (BLMs) have been developed. However, applicability of nickel BLMs to Japanese river waters has not been tested sufficiently. In this study, chronic toxicity of nickel in Japanese river waters was examined by using *Daphnia magna* to examine whether the BLM for chronic toxicity of nickel to *D. magna* could apply to Japanese natural waters. We collected 4 different water-quality type of Japanese river waters in terms of DOC and hardness and then obtained 3 pH-level waters for each of the 4 river waters by adding MOPS and NaOH or HCl. We conducted chronic toxicity test of nickel in each of 12 river waters by using *D. magna*. The predictive capacity of the BLM for chronic toxicity of nickel to *D. magna* was evaluated by using results of toxicity tests. Based on results, applicability of the biotic ligand model to Japanese river waters and development of the models for Japanese river waters are discussed in the presentation.

TUPC17

The effect of aqueous potassium concentrations on thallium-induced toxicity in medaka (Oryzias latipes) larvae

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Thallium (Tl) is a trace element commonly used in the electronic, optical and semi-conductive industries etc. With the booming of these high-tech fields, Tl usage has rapidly increased, leading to frequent detection of Tl in the aquatic environment due to various routes of human activities. Tl mainly exists in the environment as monovalent Tl (Tl^+) and it is a priority pollutant of high toxicity suggested by the US Environmental Protection Agency and the European Water Framework Directive. However, the information regarding toxic mechanisms of Tl^+ is currently limited, so the associated risk of Tl^+ exposure and toxicity to the aquatic ecosystem is currently unclear. Since ionic radius of Tl^+ is similar to that of potassium (K^+), Tl^+ may interact with K^+ ion channels(e.g. Na^+/K^+-ATPase) and interfere with its bioavailability and metabolic processes, thus causing toxicity in aquatic organisms. In this study, we used medaka (*Oryzias latipes*) larvae as a model organism to investigate the chemical and biological interaction of Tl^+ versus K^+ to aquatic organisms. We aim to assess the agonistic potency of Tl^+ versus K^+ to compete for the Na^+/K^+-ATPase in synthetic natural water of various K^+ concentrations and associated toxic mechanisms. The mortality, behavioral analysis and gene expression were used to assess the acute and sublethal neurotoxic effects. Our results showed that Tl^+ induced higher mortality to medaka larvae in dosing solutions containing lower K^+ concentrations than those with higher K^+ concentrations. After a 7 day-exposure, the locomotor activity of larvae treated with sublethal concentrations of Tl^+ was significantly altered. The average velocity of larvae exposed to 50 µg/L Tl^+ significantly decreased when compared to the control group, while the absolute turn angle of treated larvae significantly increased in a dose-dependent manner. Additionally, the mRNA expression of genes for nerve function and development including Na^+/K^+-ATPase activity, myelin basic protein and synapsin IIa significantly increased after Tl^+ exposure. However, the expression of α-tubulin was decreased. The activities of antioxidant enzymes such as catalase and glutathione S-transferase were also inhibited by Tl^+ after 7 day-exposures, indicating an induced oxidative stress in larvae. Overall, this study showed a potential neurotoxic effect of Tl^+ and itsassociation with different K^+ concentrations of dosing solutions in medaka larvae. The detailed toxic mechanisms of Tl^+ require further investigation.

Revision of the EFSA Guidance Document on Birds and Mammals (PC)

TUPC19

From seeds to plasma: confirmed exposure of multiple farmland bird species to a systemic pesticide in the field

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Many farmland bird species have a high proportion of crop material in their diet, and as a result are at risk of being exposed to systemic pesticides via direct ingestion of either treated seed or crop seedlings. Current risk assessments assume that the majority of seeds will be buried after sowing and are therefore inaccessible to birds, whereas the amount of residue in crop seedlings as a result of systemic seed treatments is largely unknown. Here, winter cereal treated with the neonicotinoid clothianidin is used as a model system to test the validity of these regulatory assumptions. In 2015 and 2016, surface seed densities were recorded post-sowing, and crop seed and seedling samples were collected at multiple time points across 25 sites (East Anglia, UK) sown with neonicotinoid-treated seed. Bird abundance and observations of birds consuming treated seed were also recorded. In 2017, plasma samples were obtained from 11 farmland bird species across six sites (Lincolnshire, UK) sown with neonicotinoid-treated seed, pre- (controls) and post- (treatment) sowing. Seed, seedling and plasma samples were analysed for clothianidin via liquid chromatography-mass spectrometry. Surface seed densities were greater at headlands compared to field centres; median clothianidin residue was 181,510 ng/g wet weight in seeds (n = 109) and 1,131 ng/g wet weight in seedlings (n = 88). Clothianidin residue detected in seeds decreased over time, with the steepest decline occurring three days after sowing. Overall, 71 bird species were recorded in treated fields, 15 of which were observed consuming treated seed. In total, 37 out of 73 treatment samples (10 out of 11 species) and four out of 37 control samples (2 out of 11 species) tested positive for clothianidin. Median clothianidin residue in plasma samples was 10 ng/mL (n = 41) and the largest concentration (69,300 ng/mL) was collected from an individual yellowhammer (*Emberiza citrinella*). Results suggest that exposure to neonicotinoids occurs across multiple species of UK farmland bird, and that treated seeds are readily accessible to a range of bird species at field headlands sown according to normal agricultural practice. This study provides evidence that dietary exposure of farmland birds to insecticide seed treatments may be underestimated by current risk assessment procedures, with potential implications for non-target avian species worldwide. \n

TUPC20

Bird nest monitoring studies: standardisation of study designs for the revision of the EFSA Guidance document

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For the assessment of the risk from Plant Protection Products (PPPs) to birds, the official guidance in the EU (EFSA Guidance Document 2009, GD) indicates that one option to demonstrate acceptable risk is to conduct so-called field effects studies. Such studies are desirable as they take into account all routes of exposure as well as the natural variability. Despite these advantages, their acceptance by member states varies, because the actual GD gives recommendations but no detailed information about the required test design of such studies. Our work contributes to the standardisation of nest monitoring protocols in order to enable comparisons between individual studies. The aim is to provide a framework regarding study area selection, proper study conduct, relevant parameters and suitable statistical tools. The design of nest monitoring studies should include endpoints which are considered in the laboratory avian reproduction tests according to OECD or EPA: number of laid eggs, infertile eggs and hatched eggs, embryo development, weight of chicks at the age of 8 or 14 days depending on the species and survival of chicks. Unlike the tier1 tests, nest monitoring studies additionally cover the period of parental care of the chicks after hatching, a phase of great importance for altricial species like most European farmland birds. Furthermore, the high degree of environmental standardisation in animal lab studies can contribute to spurious findings and little external validity (Richter 2009). In comparison, nest monitoring studies provide the most realistic tool if they are appropriately designed for taking into account variation in environmental conditions (weather, food availability etc.). It should be noted that the absence of observed sublethal effects does not indicate a failure of the study design, as field effect studies do not aim to establish a dose-response relationship. What they can provide, with the help of appropriate statistics, is the assessment of the impacts of relevant environmental factors (including PPPs) on reproduction, in spite of the high natural variability. With a more detailed guidance, field nest monitoring studies can be a valuable tool in the assessment of the PPP risk to birds, as well as part of a post-registration monitoring. To accomplish this, agreed standards should be defined in the context of the revision of the EFSA GD on birds and mammals.

TUPC21

Home range and habitat use by two farmland birds in cereal landscapes in Central Europe - results from industry studies

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For the assessment of the risk to birds from Plant Protection Products (PPPs), the official guidance of the EU (EFSA guidance document), gives the option to refine exposure estimates by conducting generic field studies, which provide data on the use of specific crops by particular species in different regions and seasons. At the request of industry, several studies have been carried out based on radio-tracking, a highly accurate methodology for the monitoring of a relevant number of individuals. These studies are primarily used to estimate the Proportion of an animal´s daily diet obtained in a Treated habitat (PT). However, the scope of the studies reaches further, since they contain also a wealth of further information that allows accurate assessments of the home ranges and habitat preferences of birds in agricultural environments. Here, we will present the insights of three radio-tracking studies on two bird species (skylark and yellowhammer) in agricultural areas with predominance of cereals during the drilling season in autumn. The selected species are typical farmland inhabitants, mostly granivorous during the autumn/winter and yet with contrasting ecologies. Radio-tracking was performed as continuous sessions over the daily activity period or by temporary checks over the study duration, and habitat mapping was carried out at a spatial scale from single bird home ranges up to hundreds of hectares of the study area. Both species showed their habitat preferences clearly, agricultural landscapes with open areas for the skylark and mixed agricultural landscapes with vertical structures (hedges, small forests) for the yellowhammer, as well as differences in the preferred foraging crops. Individual home ranges differed considerably and were in general substantially larger during the full duration of the studies, showing inter- but also intra-individual variation that indicates high flexibility in the individual behaviour. Information extracted from this data may allow for better evaluations of the use of relevant agricultural landscapes by farmland bird populations. It could strengthen the risk assessment of PPPs and therefore should be taken into account in the ongoing revision of the EFSA GD. Furthermore, data obtained in these studies on behaviour, spatial and temporal habitat use and feeding ecology of farmland species provide detailed knowledge of general interest for ornithologists, conservation scientists and experts in agri-environmental policy.

TUPC22

Calculating realistic long-term PT values for wildlife risk assessment - insights from telemetry field studies

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The current EFSA guidance document on risk assessment for birds and mammals assumes, if no specific data is available, that animals feed exclusively on pesticide-treated fields. One important recommended and frequently used refinement of the risk to birds and mammals from pesticide exposure is a more realistic estimation of the "portion of food animals obtain from treated fields". This so-called PT factor is equalized (according to EFSA B&M Guidance Document 2009) as the portion of time an animal spends potentially foraging over the course of a day within potentially treated fields, i.e. within the respective crop of interest. Radio-tracking is the best method to generate PT values, for long-term risk assessments. Due to time and resource constraints, such empirically recorded radio-tracking data are typically restricted to single or few daily activity PT values per tracked animal, and do not cover long-term periods (e.g. 21 days). Accordingly, intra-individual variability in daily PT values over an extended time period can often not be taken into account for the calculation of a long-term PT. Through analyses of multiple field studies, we further assess the applicability of a Monte Carlo probabilistic approach to simulate 90th percentile 21-day PT values, as presented in Ludwigs et al. (2017). We analyse different original and relevant radio-tracking data sets, which cover a range of different numbers of individuals and sessions per individual of common farmland species like skylarks, wagtails and brown hare. Our general approach of modelling 21-day PT values thus covers intra- and inter-individual variability of farmland species foraging in cropped habitats. We demonstrate how the different data sets from telemetry field studies can result in more appropriate 21-day PT values for use in pesticide risk assessment. These modelled PT values are compared to the current regulatory standard within the evaluation process, where PT refinement is typically restricted to the 90th percentile of the empirical data. It is proposed that this method is considered in the revision of the current EFSA B&M Guidance Document (2009).

TUPC23

Distinctive patterns in the cumulative probability distribution of PT values of several bird species. Significance of the 90th percentile - How to reach a realistic PT value for long-term exposure?

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Refinement steps in the higher tier risk assessment include the PT value, defined as the Portion of diet obtained from a Treated area and ranging from 0 (diet portion= 0%) to 1 (diet portion = 100%). EU regulatory authorities recommend the 90%ile of the measured PT data to be used in the refinement. However, using this single value only neglects valuable information about the studied bird populations. We analysed the cumulative frequency distributions of PT values of several species in the same crop type (orchard or cereal) in two countries (ES and UK). Interestingly, three types of distribution patterns represent the relevance of the crop for the studied population, respectively. Type I distribution follows a quadratic function, where the 90%ile of the PT value is representative for the population. For example, blackbirds in citrus orchards show a 90%ile PT (= 1) close to the median (0.9) and is thus representative for this species in this habitat. Type II follows a linear function where the 90%ile represents half of the population. Type III follows a root function, where for the majority of individuals the actual PT is much lower than the 90%ile, so the crop is the feeding habitat of only few individuals. For example, tit species in pome fruit orchards show a great difference between the 90%ile PT (0.74) and the median (0.19). In the case of type III distributions the 90%ile PT does not describe the foraging behaviour of the population in the crop appropriately. These insights are important, since the 90%ile is used for long-term risk assessment as a representative value of the **mean** exposure of 90% of the population over a longer period (e.g. 21 days). This assumption would only be correct if individuals with a 1-day PT value around the 90%ile were similarly exposed every single day in the whole period. Instead, both literature and our own data shows that PT values of the same individual differ considerably among days. Thus, over any given period of time, the PT of 90% of the population is clearly lower than the 90%ile of 1-day, especially when the frequency distribution follows type II or type III. In such cases, a realistic 90%ile PT can be estimated based on repeated sessions of individuals. As long as no such data is available, the 90%ile should be reassessed by taking into account the difference to the median. This approach, validated by published data of repeated individual sessions, gives a more appropriate PT value for long-term risk assessments.

TUPC24

The Minimum detectable differences - a way to estimate the power of a small mammal field effects study a posteriori

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Before conducting a field study it is sensible to find out the number of fields necessary to find a specified effect, so that the study can be planned accordingly. This power analysis requires reliable data from previous or pilot studies that provide the necessary means and standard deviations. For field effects studies these data often does not exist. The Minimum Detectable Differences (MDD) can be used to calculate the smallest detectable effect of the study *a posteriori.* The calculation designed for a t-test by Brock et al. (2015) can be modified for statistical methods adequate for analyzing field study data such as Generalized Linear Mixed Models (GLMMs) (Peters et al., 2016, Supporting Information). One possible example is a field effects study focussing on the influence of a Plant Protection Product (PPP) application on common vole populations. For the common vole it is especially difficult to get reliable standard deviations as the voles undergo multi-annual population cycles with huge differences between low and peak years. The guidance document for aquatic organisms by EFSA (2013) provides a ranking with four MDD classes. The best achievable class is class IV with MDD < 50% and defined as “Small effects can be determined statistically”. This < 50% threshold was used to assess the MMD% for four field data sets as there is no such ranking for terrestrial field effects studies provided by EFSA or any other regulatory authority. All together MDD% for 31 trapping sessions were calculated with only one session above the 50% threshold and a mean of 18%. Compared to a 50% threshold for aquatic studies this is a very acceptable result. To get an idea what this difference means in common vole populations, the deviation from the mean between control site populations within the four example studies was calculated. These differences aggregated to values between 17 and 110%. Compared to this natural variation in population density the detection of an average of 18% difference between treated and untreated populations is a promising result.

Can We Demystify Machine Learning for Environmental Chemistry and (Environmental) Toxicology? (PC)

WEPC01

Advancing machine learning in environmental toxicology via information sharing and transfer learning

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In early development of Machine Learning (ML) and Artificial Intelligence (AI), there was a strong skepticism on how to interpret the prediction results, and how to generalize the results to more complex situations. To respond to the skepticism, more data such as images and texts were collected and shared in standard formats to strengthen the training of the ML/AI systems. Through extensive training on wide variety of sharable data, many systems such as IBM Watson or Google Alpha Go nowadays can outperform human precision in many fields. In toxicology, if wide variety of data can be shared in standard formats, we can train the ML/AI systems to generate high-confidence hypotheses to guide toxicologists focusing their studies on significantly smaller subsets of targets. Luechtefeld’s 2018 study echoed the similar vision, and analysts in other fields have been using the similar approach in guiding their search for the bank or medical frauds. Sharing datasets in standard formats can also allow scientists to train more generalizable and reliable ML/AI systems. For example, over years of collecting videos in standard formats, self-driving cars can now recognize complex driving conditions with high precision. Similar but limited successes in toxicity prediction have been reported in *DeepTox* and *InSilico*. We can greatly expand the successes to broader applications with larger sharable datasets. For the interpretability issue, many techniques have been developed to help users to identify important features that lead to certain ML/AI predictions. The reliability of such feature identification methods can be improved with wide varieties of large datasets. For example, the LASSO method can compute the positive or negative weights of each predictor in influencing the prediction results. The “attention” method also has potentials in identifying the important “toxicophores” that lead to toxicity or disease. As discussed above, large-scaled information sharing in standard formats will enable scientists to train more sophisticated ML/AI systems that are more generalizable and reliable. More importantly, the trained systems can again be shared and quickly be retrained using much smaller datasets by other scientists for different but related toxicology analyses. This new promising concept of ***transfer learning*** is our current research focus. We plan to expand the AOP prediction systems that we presented in recent SETAC meetings and make those transferable to other toxicology studies.

WEPC02

A machine learning approach for (transcript-)omics data in ecotoxicology to improve consistency of microarray sample sets of chemically treated fathead minnows

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Omics technologies are applied more and more in ecotoxicological research. One challenge in omics research is to establish consistency between different studies. The present study investigated, whether comparability of microarray data sets can be improved using a machine learning algorithm. Nineteen GEO-sample sets were used to train an universal ecotoxicogenomic map for hepatic tissue of adult male fathead minnows exposed to either waste water or single chemicals or mixtures of chemicals. The unsupervised learning approach reduces the dimensionality of the data set without losing information by clustering genes to nodes according to their similarity in expression behavior. The 54 projections on the ecotoxicogenomic map showed unique patterns. Nevertheless, it is possible to group reduced profiles by their similarity of patterns. In the data sets of an interlaboratory study, a common EE_2-pattern could be identified with 12% of significantly regulated nodes in common and a mean pairwise overlap of 62%. In comparison, We could show that differential expression of microarrays across six different laboratories is more congruent using the application of a machine-learning approach than using single genes. A combined filtering for differential expression of nodes ($p < 0.05$; $|logFC| > 1$) improved consistency in comparison to the results within the interlaboratory study (5% total overlap and 59% mean pairwise overlap). A further coarsening of transcriptomic units by hierarchical clustering as second reduction to the ecotoxicogenomic map, enabled us to determine a combination of estrogenic specific differentially expressed units in this interlaboratory study and two further profiles of microarray experiments that examined estrogenic treated fathead minnows.

WEPC03

Computational tools for in silico multi property profiling of chemicals

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In the last decade the high economic and social costs of experiments necessary to support the timely identification and management of potential risks associated to chemicals, has pushed the use of *in silico*, i.e. computer-based, alternatives to animal testing, onwards. Machine learning methods based on linear and non-linear approaches as well as data mining techniques have been widely applied to this end. Nowadays thousands of *in silico* models based on structure-activity relationships (i.e. QSARs) are available to predict different endpoints of interest for the scientific and the regulatory community. OECD and ECHA guidelines provide support for the correct development, validation and application of these tools, however not all models are equally transparent, interpretable or have a clearly defined domain of applicability. These issues may limit the application of these models as well as negatively affect the potential benefits associated to the use of alternatives to animal testing. This is particularly important considering the large amount of resources invested worldwide over the last years for the development of these alternative approaches. In the last 20 years, our research group has developed many validated QSARs to predict different physico-chemical properties and biological activities useful for the hazard assessment of legacy chemicals and contaminants of emerging concern, using linear and non-linear approaches. The experience gained in these two decades of research was used to develop the software QSARINS, which is suitable for experienced model developers, and the recently released QSARINS-Chem, for models application only, which is dedicated to all users, i.e. with or without previous modelling experience. In this presentation, we provide step-by-step examples of how these tools can be transparently used to generate multiple linear regression models as well as predictions for various endpoints. The proposed results show that these tools are useful to fill data gaps and profile/prioritize chemicals according to multiple endpoints of interest, also in combination with predictions generated by other software and literature models.

WEPC04

Graph edit distance as molecular similarity measure for virtual screening

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Graph edit distance has been used for more than 40 years to compare objects in machine learning when these objects are represented by structures, such as attributed graphs, instead of vectors. In some applications, attributed graphs have more ability to represent the intrinsic properties of the elements than a vector of global features. This is because the local parts of the object can be explicitly represented by nodes and edges. Given two graphs, the graph edit distance between them is defined as the minimum number of modifications that are required to transform one graph into the other. To do so, it is needed to define these modifications, which are called edit operations. Basically, six edit operations are defined: insertion, deletion and substitution of both nodes and edges. Edit costs are defined to quantify the amount of distortion that each edit operation introduces. Thus, they are selected according to how similar the nodes and edges are. For instance, it is logical to think that the cost of substituting a hydrogen-bond donor with a joint hydrogen-bond donor-acceptor should be less penalized than the cost of substituting a hydrogen-bond donor with an aromatic ring system. In chemistry, attributed graphs (for instance *reduced graphs*) have been used to represent chemical structures using pharmacophore-type node descriptions to encode the relevant molecular properties. Nevertheless, the comparison has always been carried out by first converting the graphs into a vector of elements (for instance *fingerprints*) and then comparing the vectors using *Tanimoto distance*. In this paper, we propose to directly compare the reduced graphs through the graph edit distance, with the aim of keeping the structure and local information of the compared compounds. In the experimental evaluation, our method is applied to ligand-based virtual screening applications, where an estimation of bioactivity for a chemical is provided based on the bioactivity of similar compounds. Results obtained for graph edit distance show to have a better performance than other previous methods used on reduced graphs, using array representations. Overall, it is shown that reduced graphs along with graph edit distance is a widely applicable combination of methods, capable of identifying bioactivity similarities into a structurally diverse group of molecules.

Advances in Soil Ecotoxicology and Risk Assessment - Impact, Ecotoxicity Tests, and Concepts for a Retrospective Environmental Risk Assessment (PC)

WEPC07

A new method for evaluation of soil ecological toxicity using vibration sensors and earthworms

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The methods suggested by OECD and ISO have been widely used for evaluation of soil ecological toxicity (SET), but their major disadvantages are to focus on only toxic substances and to requrie a relatively large amount of soil samples and large numbers of target species. To overcome those problems, this study conducted to develop a new method via detecting the movement of earthworms using vibration sensors. In order to establish the new method, the various conditions were optimized in terms of soil properties, biological aspects, and device specifications. After optimization, the method was verified by the evaluation of SET using soils contaminated with zinc (Zn). In optimization experiments using clean (uncontaminated) soils, the effects of soil properties [water holding capacity (WHC) and degree of consolidation (DOC)], the number of earthworms used, monitoring time and duration, and device specifications (shape and size) were investigated. On the other hand, in verification experiments using optimized conditions, the farm soils were tested after artifically contaminating with Zn concentrations. Based on the results of optimization experiments, 50% and 1.09 were investigated be most relevant for WHC and DOC, respectively, with consideration of movement and weight change of earthworms. The larger the number of earthworms used, the more frequent movement of earthworms detected. The height and radius of device were proven to be another crucial factors controlling the performance of the method. After testing the efficiency of several heights and radii, the optimal height and radius were proposed 5 cm and 6–10 cm, respectively. In addition, the leave and movement of earthworms decreased with increase in monitoring duration, and it might be caused by detrimental environments on vibration of soil particles and feeding of earthworms as a result of decreasing WHC. Accordingly, the relevant monitoring duration was proposed to be 3~6 days. The results of experiment on verification suggest that the movement of earthworms was decreased with increase in Zn concentrations, and it could be attributed to that earthworms minimized dermal contact with contaminated soils and the quality of soils as feeding media were degraded. Based on the detected movement of earthworms, EC50 was estimated and it was very similar to existing ones. Consequently, the movement of earthworms detected by vibration sensors can be applied to evaluate SET.

WEPC08

Terrestrial ecotox studies with difficult substances - A comparison of different application techniques

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The OECD Guidance document no. 23 identifies different classes of so-called difficult substances. Dependent on the physico-chemical properties of these substances, non-standardized test designs are presented and recommended. No such guidance is available for terrestrial ecotoxicity studies in soil although testing of different classes of chemicals and supporting analytical measurements become ever more important. We compared different techniques of soil application for three representatives of difficult substances. All combinations were supported by analytical measurements of the spiked soil. The analytical results were compared and discussed regarding recovery in soil, homogeneity of distribution and the overall usefulness of the different application techniques for difficult substances.

WEPC09

New soil test method for assessing contaminants using oribatid mites - Results from an international ring test

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The use of oribatid mites has been steadily increasing in the assessment of chemical substances in soils, in addition to contaminated site soils. Oribatid mites represent a unique ecological niche within terrestrial systems, are among the most abundant species within surface soils, contributing to nutrient cycling through litter break-down. Efforts within the last several years have led to the development of a new test method within Environment and Climate Change Canada, using the oribatid mite species, *Oppia nitens* (CL Kock, 1839). The new method describes a 28-d test, using age-synchronized test organisms amenable to assessing the effects on adult mortality (if present) and juvenile production. Furthermore, efforts have also been directed to the inclusion of this new test method as part of the International Organization for Standardization (ISO) series of standards within Technical Committee ISO/TC 190 for Soil Quality. As part of standardization efforts, an international ring test was completed, involving 8 participating laboratories from Canada and Europe. Three rounds of testing were completed: (i) performance testing in artificial (AS), agricultural (VSL) and forest soil (FS) (for laboratories to become familiar working with the test species) (Round 1); (ii) a chemical test using boric acid in a field (VSL) soil (Round 2); and (iii) a chemical test using boric acid in the standard LUFA 2.2 test soil (Round 3). The results of the ring test will be presented from all three rounds, wherein, together with performance test data conducted by ECCC Canada using 13 soil types and horizons, the test validity criteria for this species and test method were finalized. The new test method is the first standardized effort for oribatid mites; the method will complement an existing suite of soil test methods (e.g., earthworms and collembolan), with applicability to both forest and agricultural soils.

WEPC10

Soil metal-contamination meets global climate change: effects on soil properties and toxicity risks to soil biota

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Anthropogenic activities have been inducing alterations on Earth's climate system. The Intergovernmental Panel on Climate Change (IPCC) predicts major changes towards the 21st century (e.g., higher frequency/intensity of floods and droughts; increasing global air temperature). These alterations may impair the biotic and abiotic components of terrestrial ecosystems, affecting their functioning and sustainability and thus the services they provide. This scenario may worsen in anthropogenic-degraded areas where soil biota has to deal with already multiple stressors (i.e., multi-stressed environments) and where the behaviour/distribution of the contaminants present may change depending on the prevailing climate conditions and therefore their toxicity too. So far most of the studies dealing with climate change and soil toxicity risks have been focused on the effects of single climate factors on chemical-spiked soils, while less attention has been paid to climate factors combinations and/or anthropogenic-degraded field soils. In order to overcome this issue a new research line has been created in the Dept. Biology & CESAM from Aveiro University (Portugal) through the performance of the following research projects: GLOBALTOX (Marie Sklodowska-Curie programme; 704332), METOXCLIM (FEDER, COMPETE 2020 and Portuguese FCT; POCI-01-0145-FEDER-029557) and MICROCLIM (French CNRS/INEE via OHMI). The overall goal of the research line is to assess how biotic and abiotic components of field metal-contaminated soils may be affected under the current global warming perspective, by using toxicity (measured by means of soil invertebrates) and the associated changes in functional soil parameters (physico-chemical, microbiological) as indicators of ecosystem services. A inter/multi-disciplinary ecotoxicological approach is being applied to field metal-contaminated soils, using different invertebrate species and expositions to single/multiple climate factors (soil moisture content, air temperature, UV radiation and atmospheric CO_2). Climate factor combinations are be based on IPCC future climate predictions. The research line considers changes in key soil parameters (e.g., pH, organic matter, metal availability, microbiology) and soil invertebrates (e.g., body metal content, enzymatic biomarkers, gene expression) to understand the effects at organism/population level (e.g., survival, avoidance behaviour, reproduction).

WEPC11

BioNem Mulch Project - Assessing the efficiency and non-target effects of bionematicide delivery systems

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The use of synthetic nematicides is one of the most frequent and efficient strategies to control plant-parasitic nematodes (PPN). Root knot nematodes (RKN, Meloidogyne spp.) are among the PPN with higher economic impact worldwide, as they affect crop productivity, quality and value. However, due to increasing concerns on the risks posed by chemical nematicides, the development of environmentally friendly bionematicides has been stimulated. Recent studies have demonstrated that several phytocompounds have nematicidal activity but their applicability on RKN management should be explored. Currently, mulching plants with polyethylene plastic is a common practice to manage weed/soil-borne plant pathogens, although the waste disposal of such non-biodegradable mulches raises several environmental problems. The BioNem Mulch project, comprises a multidisciplinary approach and focuses on the development of fully degradable-in-soil bionematicide delivery systems (BDS) for RKN management on tomato crops. More specifically, this project aims to: 1) evaluate the impact of nematicidal compounds, in tomato crop and RKN life cycle; 2) develop BDS based on biodegradable plastic mulches and bionematicides; 3) assess BDS biodegradability and efficiency on RKN management; and 4) evaluate the effects of BDS field applications on non-target soil mesofauna. Soil quality indicators, such as mesofauna diversity (e.g. non-target nematode and microarthropod communities), microbial functional parameters (microbial biomass and soil basal respiration), and BDS degradation will be analyzed, under pot and field scenarios, to fully monitor the associated soil impacts.

WEPC12

Comparing effects of fludioxonil on non-target invertebrates using ecotoxicological methods from single-species bioassays to model ecosystems

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Lower tier toxicity testing used for risk assessments of plant protection products (PPPs) is conducted with single species. Informations from such toxicity tests are, however, limited to direct effects of the respective tested substances. Although the uncertainties regarding the protectiveness of these tests for *in-situ* communities are known, informations on effects of PPPs on community-level of non-target organisms are scarce. Model ecosystems, i.e. microcosms, are suitable to bridge this gap between single-species tests and field studies since they provide controlled experimental conditions and are able to demonstrate direct and indirect effects of the respective substances. In the present study, single-species toxicity tests and soil-spiked microcosms were used to comparatively investigate the toxicity of the non-systemic fungicide fludioxonil (FDO) on non-target soil organisms, i.e. nematodes. Regarding soil invertebrates, nematodes are among the most abundant metazoan organisms and are considered as important components of the soil food web. Since nematodes are suitable for risk assessments via various assay tools ranging from single-species toxicity tests to field studies, potential effect of FDO on these non-target organisms were assessed using standardized toxicity tests with *Caenorhabditis elegans* (ISO 10872) in spiked soil exposure and *in-situ* nematode communities, sampled from microcosms with FDO-spiked soils. In the standardized toxicity tests, FDO inhibited the reproduction of *C. elegans* dose-dependently, with a chronic 96-h EC50 of 209.9 mg FDO/kg soil dry weight (dw) and a 96-h No Observed Effect Concentration (NOEC) of 63.0 mg/kg soil dw, which is comparable to the 28-d NOEC of *Chironomus riparius* in sediment (40 mg/kg sediment dw) and within the range of the 56-d NOEC of *Eisenia fetida* (20 mg/kg soil dw) and 28-d NOEC of *Folsomia candida* (125 mg/kg soil dw). In the spiked microcosms, distinct effects on *in-situ* nematode communities could be measured, with significantly lowered abundances in spiked soils, (40% and 50% reduction of nematode abundance in soils spiked with 259.3 and 476.4 mg/kg dw. Overall, this study provides new insights into the impact of the non-systemic fungicide fludioxonil on non-target soil organisms and demonstrated the general suitability of standardized toxicity testing on *C. elegans* in protecting *in-situ* communities.

Recent Approaches in Establishing Linkages between Exposure Science and the Environmental Effects of Trace Organic Contaminants (PC)

WEPC13

Monitoring estrogenic activities of waste and surface waters using an in vivo zebrafish embryonic assay: comparison with in vitro cell-based assays and determination of effect-based trigger values

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Monitoring of environmental estrogens has become a major concern to assess the quality of water bodies. In that respect, the use of *in vitro* reporter gene assays to monitor estrogenic activity has proven relevant as it enables an integrative and quantitative assessment of ER-active contaminants. However, *in vitro* cell assays may not necessarily reflect the estrogenic activity observed *in vivo* in fish, raising the question of the relevance of *in vitro* measurement of estrogenic activity to predict risk for aquatic species and ecosystems.In this context, our objective was to determine 1) whether estrogenic activity measured *in vitro* led to an *in vivo* response in a fish biological model, and 2) on the basis of such an *in vitro* / *in vivo* comparison, whether there exists an *in vitro* threshold value above which an *in vivo* effect is detected/evidenced. This study reports the use of the zebrafish-based EASZY assay to assess *in vivo* estrogenic activity of 33 surface (SW) and waste water (WW) samples collected across Europe and that were previously well-characterized for estrogen hormones and *in vitro* estrogenic activity (Könemann et al., 2018). We showed that 18 out of the 33 SW and WW samples induced estrogenic responses in transgenic zebrafish embryos leading to significant and concentration-dependant dependent up-regulation of the ER-regulated cyp19a1b gene expression in the developing brain. The in vivo 17β-estradiol-equivalents (EEQs) were highly correlated with both the chemical analytical risk quotient (RQ) based on steroidal estrogen concentrations and EEQs reported from 5 different in vitro assays. Based on in vitro vs. in vivo comparison, regression analyses allowed to determine for each in vitro assay an optimal cut-off value which was defined as an effect-based trigger values (EBTs) above which in vivo responses were observed. The definition of these *in vitro* assay-specific EBTs values allowed to improve the sensitivity and specificity of the individual *in vitro* assays for predicting a risk associated with substances acting through the same mode of action within environmental mixtures. Altogether, this study provides experimental demonstration of the toxicological relevance of in vitro-based assessment of estrogenic activity and recommends the use of such *in vitro/in vivo* comparative approach to refine and validate EBTs for mechanism-based bioassays.

WEPC14

Combined analytical and effect-based monitoring of EU Watch List water samples

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Three steroidal estrogens, 17α-ethinylestradiol, 17β-estradiol, and estrone, and the cyclooxygenase (COX) inhibitor pharmaceutical, diclofenac have been included in the first Watch List of the Water Framework Directive. This means that more Union-wide monitoring data are required on these potential water pollutants before they can be considered for inclusion in the list of priority substances. However, it is a challenge to detect these chemicals at bioactive concentrations (low pg/L) with existing routine analytical methods. Effect-based methods are promising bioanalytical tools that could circumvent such detection challenges by measuring total estrogenic activity or COX inhibition in the aquatic environment in a cost-efficient way at very low concentrations exerted by the above mentioned substances. To investigate the applicability of effect-based methods for monitoring specific groups of substances under the WFD a monitoring project was initiated by the Swiss Centre for Applied Ecotoxicology (CH) and the German Environment Agency (DE). Furthermore, the project aimed at providing high-quality monitoring data for these Watch List substances. Surface waters from Watch List sampling stations in 14 EU member states and 4 Swiss cantons were analyzed for steroidal estrogens and diclofenac by LC-MS/MS and screened *in vitro* for estrogenicity by the human cell-based ERα-CALUX and the yeast estrogen screen, AYES, and COX inhibition. The presentation aims to summarize the major results and conclusions of this large monitoring project: All together high-quality monitoring data were produced and the applicability of effect-based methods was shown to complement targeted monitoring of specific groups of substances in the aquatic environment under the EU WFD. The applied effect-based method of steroidal estrogens is more sensitive than chemical analytics pointing out its good screening potential. At the same time, it also implies that analytical methods are faced with detection challenges of the target analytes at low concentrations. Chemical analytical and effect-based methods can be combined in order to get the benefits of both: to receive indication on substances and their contribution to the measured effects, to study variable chemical exposure, to exclude false negative measurements, to monitor specific groups of substances in the aquatic environment.

WEPC15

Identification of chemicals in contaminated sediments with the support of bioassays

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Over the last decades, an increasing number of pollutants have been detected in the environment. Even at trace levels, certain of these contaminants pose an increasing environmental, human health and economic risk. The identification of toxicants is a crucial step to protect aquatic environment and is an on-going challenge that may be addressed by toxicity profiling for environmental quality assessment. The Baltic Sea has been exposed to an extensive use of chemicals from the very beginning of the industrialization of the region and its marine environment has one of the longest histories of contamination in the world. The city of Sundsvall has an industrial history of over 150 years, associated with major pulp and paper industry as well as chemical and aluminum manufacturers. As a result, coastal sediments in the area are polluted by trace metals and organic compounds of industrial origins. *Aim*: Assessing the toxicity of the sediments in Sundsvall area and identifying chemicals responsible for the observed effects. *Methods:* Sediment samples were taken in connection to the different industries located in the Sundsvall Bay (river estuary S1, fiber bank S2, oil depot S3, primary aluminium producer S4, residential area under the influence of the aluminium factory and river current S5, wood finishes and surfactants production S6 and further away residential areas S7 and S8). The battery of bioassays includes, mutagenicity with different strains of Salmonella, fish embryo toxicity, DR-CALUX (total extract and purified extract), ER-CALUX and TTR-binding. The chemical identification is done by GCxGC-HRMS and LC-ion mobility-HRMS. *Results:* Plausible presence of amino compounds in all sediments samples, except for S1 and for S2 (fiber bank sample is cytotoxic). In general, samples S2, S3 and S4 (fiber bank, oil depot and aluminium production) exhibit most of the positive responses towards the different bioassays. The results for the fish embryo assay show mainly malformation when exposed to S2. In details, nitro-PAHS are expected in samples S3, S4 and S5, high amount of PAHs in S4 and compounds such as PCBs, dioxins and furans can be present in S3. Possible presence of estrogenic active compounds in S2, S3 and S4 (e.g. natural and synthetic steroids, surfactants, pesticides …). Finally, compounds like OH-PBDE, triclosan, nonylphenols or musk for instance could also be present in S2 and S4.

WEPC16

Successfully interlinking temporally- and specially- explicit fate and effect modelling within an agricultural field

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The development of efficient and sustainable molecules for plant protection is characterized by extensive requirements for the environmental risk assessment. Proof of efficiency is part of the risk assessment procedure and can influence the development and registration process for new molecules and products. The proposed placement strategy (application type and application rate) derived from efficacy trials and the resulting environmental load (driving the Predicted Environmental Concentration (PEC)) are the basis for the risk assessment for non-target organisms. Particularly for substances used to control root damaging pests, the assessment of efficacy is demanding and costly due to the complex interplay of system components and the limited options for investigating interaction between roots, pests and substances without disrupting the system. Here we present a new temporally- and spatially-explicit modeling concept called COMPASS (Comprehensive Model for Pesticide Activity in SoilS) that simulates the interaction between roots, pests and substances in the soil profile and allows investigation of the efficiency of different placement strategies. The modeling concept consists of an individual based population model for the pest, an agent based root growth model that can be affected by the pest, a water and substance transport model that is linked to the root growth model, and a toxicokinetic/toxicodynamic model that converts individual exposure of moving organisms in the soil profile to their survival. The concept was developed using the corn rootworm *Diabrotica virgifera* as model species and it was evaluated using 125 field studies conducted in four regions of Illinois (USA) over a period of ten years where substance efficacy was found to be highly variable. The field studies used for evaluation of the model framework span 11 commercial products containing one of three active substances (chlorpyrifos, clothianidin, tefluthrin) and applied as seed, band or furrow application at sowing. The model simulates efficacy in agreement with the observed efficacy in 94% of all field studies tested. We discuss how a model following the new concept can be used to a) develop new molecules and products, 2) optimise application timing, application type and application rate to support integrated pest management, and c) in the future assess the interplay between control of the target pest whilst minimising risk to non-target species within a field.

WEPC17

pH-dependent toxicity due to protonophoric uncoupling

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When researching the linkages between exposure conditions and toxic effects for ionisable compounds, pH-dependence of toxicity is an important factor. One such pH-dependent effect is protonophoric uncoupling, where a weak acid shuttles protons H^+ across the inner membrane of the mitochondria, and thereby decreases/dissipates the proton gradient created by proton pumps of the respiratory chain. This gradient is essential for ATP-synthesis, and its dissipation leads to a toxic effect. Due to recent advances in the prediction of anionic permeation through lipid bilayers, we were able to develop a purely mechanistic biophysical prediction model of pH-dependent uncoupling toxicity. Our model describes a steady state situation, where the net flux of the protonophore across the membrane is zero. At the same time, we assume a toxic effect to occur once the flux of shuttled H^+ reaches the same order of magnitude as the opposing flux of H^+ created by the proton pumps. Important input factors are physiological data, like the pH- and potential-gradient across the mitochondrial membrane, as well as the fractionation (pKa) and the permeability of the weak acid. The core input is the anionic permeability of the uncoupler. At this point, our mechanistic model is well suited to predict minimal toxicities of ionisable compounds, as well as pH-dependencies and their direct cause (like ion trapping or limitations by the neutral permeability). But to avoid underestimation of toxicity due to uncoupling, heterodimer permeation will have to be included in the model. The advantage of our model over already existing quantitative structure-activity relationships (QSARs) is the ability to account for pH- or potential- gradients across the membrane. Also, the mechanistic nature of the model allows to draw direct conclusions on the factors that limit the uncoupling activity.

Disinfection Byproducts and Oxidation Byproducts: Analysis, Fate, Toxicity and Treatment (PC)

WEPC19

Toxicological Assessment of Disinfection By-products using Zebrafish Embryo Bioassays

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Disinfection of drinking water is crucial to protect human health from pathogens and to prevent their regrowth during water supply, but the reaction of disinfectant agents with organic matter can lead to the formation of disinfection by-products (DBPs). Given their widespread occurrence, the potential human health impacts and (eco)toxicity associated with exposure to DBPs are of particular interest due to their potential carcinogenicity and vary non-carcinogenic effects. Understanding the public health implications of this emerging compounds in drinking water and their unclear biological mechanism is crucial for societies, decision-makers and water safety plans. The main aims of this project are (a) to implement and validate analytical methods to detect, identify and quantify new and unregulated DBPs such as Haloketones and Aldehydes, in drinking water, in order to contribute for enhancing the effectiveness of EPAL's water safety plans and improve the knowledge on the risk of DBPs in a drinking water supply system (b) In order to assess health risk of DBPs in drinking water, a high-throughput embryo bioassay approach based on OECD 236 toxicity test with the model animal Zebrafish (*Danio rerio*) was employed. We present here the preliminary results based on the toxicological approach with zebrafish embryo bioassays, using 7 unregulated compounds, including Haloketones, Aldehydes, Alcohols and Nitrosamines. The preliminary results point to significant differences on total abnormalities, mortality and sensorimotor reflexes after 96h post-fertilization, which were compound and concentration dependent. The human health risk of the tested compounds will be discussed and integrated with the current knowledge in the field. **Acknowledgement** FCT- Fundação Ciência e Tecnologia for funding grant PDE/BDE/122649/2016

WEPC20

Assessing the occurrence of trihalomethanes, haloacetic acids and halophenols from chlorinated industrial discharges at sea and their toxicity on Paracentrotus lividus

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Chlorination, one of the most widely used techniques for water disinfection, is also considered to be one of the most effective available treatments for the control of micro- and macrofouling. However, chlorine is very reactive towards natural organic matter, ammonia and bromide present in seawater. The reactions of chlorine with these species lead to the formation of several brominated or chloro-brominated by-products, whose nature, levels and eco-toxicities are poorly documented in the literature. This study aims at evaluating the levels and impacts of chlorinated discharges in a heavily industrialized area, located in a semi-enclosed bay in South East of France. In this area, the total amount of daily releases is estimated to reach up around 6 million cubic meters of chlorinated waters. Five sea sampling campaigns were carried out between 2017 and 2019. Physico-chemical parameters were recorded on-site. Water samples were also collected using 1L bottles and stored at 4 °C after the addition of ascorbic acid or

ammonia chloride. These water samples were used for the analysis of several classes of chlorination by-products including THM, HAA and HP. Samples treatment consisted of liquid-liquid extraction with MTBE. All extracts were analyzed using GC-ECD (Clarus 580, Perkin Elmer, Norwalk, CT, USA). Limits of detection were 0.01 μg/L for THM and HP, and 0.1 μg/L for HAA. Additionally, individual and mixture acute toxicity and genotoxicity were measured on larvae of Mediterranean Sea urchin: *Paracentrotus lividus*. The toxicity test was based on abnormal growth rate to characterize the developmental effect, biochemical analysis to evaluate DNA damage and how each geographical group of urchins respond to this kind of pollution. Individual molecule toxicities were plotted on a dose-response curve, and toxicities on two population of sea urchins, either chronically exposed or not, were compared. This work showed the occurrence of THM, HAA and HP, with predominance of bromoform, tribromoacetic acid and tribromophenol in the industrial gulf. The synergic acute toxicity and genotoxicity of these three molecules on *Paracentrotus lividus* larvae has been demonstrated for concentrations significantly higher than the environmental ones, and a local adaptation was demonstrated with different molecular responses. **Acknowledgements**: This work was included in the project "Fos-Sea" funded by the French Research Agency (ANR-16-CE34-0009). *PARTICIPATION IN THE YSA*

WEPC21

Analytical method development for the determination of the emerging chlorination by-products bromobenzoquinones in seawater.

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After the first discoveries of disinfection by-products (DBPs) formation during chlorination in the 1970s, research has been conducted to understand processes of formation and identification of DBPs. Up to now, more than 600 DBPs have been identified in various water matrixes. Among them, halobenzoquinones (HBQs) were first identified and confirmed as an emerging group of halogenated DBPs in 2010. Quantitative structure toxicity relationship analysis predicted HBQs to be potential bladder carcinogens. Their occurrence in seawater has been poorly studied until now, while the potential sources of their production increase. Indeed, the brominated-HBQs may be formed by chlorination of organic matter present in sweater. They could be thus found in the marine discharges of wastewater treatment plants, in ballast waters, in the outlets of industrial plants using seawater, and in the outlets of desalination plants. Analytical methods for the determination of brominated HBQs in seawater is poorly documented in the literature. In the present study, four bromated HBQ have been analyzed, according the frequent occurrence of their chlorinated analogues in treated tapwater at ng/l levels: 2,5-Dibromo-1,4-Benzoquinone, 2.6-Dibromo-3,5-Dimethyl-1,4-Benzoquinone, 2,6-Dibromo-3-Chloro-5-Methyl-1,4-Benzoquinone, 2,3,5,6-Tetrabromo-1,4-Benzoquinone. As a first step, their stability in seawater has been studied. The results show HBQs in seawater are not stable over time, with a degradation rate of 93% in 19 hours at 20 °C for three of them.To stabilize them in seawater, it is necessary to transform them into their redox congeners, *ie* bromohydroquinones by adding ascorbic acid. To reach a low level of detection, it is then necessary to optimize a preconcentration step. In this way, we developed a new method of solid phase extraction (SPE) for these compounds by implementation of an experimental design, allowing the optimization of several factors (nature of sorbents and of solvent, extraction and elution rates).\n\nTo validate the whole analytical procedure (SPE-GC-MS analysis), real samples, coming from a heavily industrial area where 6 millions m^3 of chlorinated are discharged daily, have been analyzed. *\n Acknowledgments: This work was included in the project "Fos-Sea" funded by the French Research Agency (ANR-16- CE34-0009). M.Verlande thanks The C.R SUD-PACA and the Institut Ecocitoyen pour la Connaissance des Pollutions for his doctoral scholarship.*

WEPC22

Evaluation of the relevance of disinfection byproducts (DBPs) for the environmental risk assessment of biocides

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Disinfectants are widely used biocidal products. Their scopes of applications are quite broad and include different fields such as (drinking) water, health care, food processing or construction industry. Among the active ingredients used, highly reactive substances like chlorine, peroxides or ozone are common. During their application, numerous disinfection by-products (DBPs) are formed, especially if organic matter is present. Depending on the specific use, the reaction conditions such as presence of water, pH, temperature and application duration differ significantly and may influence the formation of DBPs. The intended use also determines possible releases of the formed DBPs into the environment. This complex situation is not adequately considered within the European biocides regulation at the moment. The authorisation of biocidal products is regulated by the EU Biocides Regulation 528/2012 which also requires the assessment of possible DBPs. The existing Guidance on Disinfection By-Products is limited to halogen-containing biocides and, selected product types and includes only general scientific strategies for the risk assessment of DBPs. A detailed European guidance for an environmental risk assessment (ERA) of DBPs is missing. On this basis a harmonized ERA of biocidal products within the EU is questionable. In order to fill this regulatory gap and develop a feasible regulatory toolkit, the German Environmental Agency (UBA) launched a research project for the consideration of DBPs within the environmental risk assessment of biocidal products (FKZ 3718 65 403 0). The project aims are a literature search based identification of DBPs relevant for the ERA of biocides and the conditions of use influencing their formation as well as the identification of active ingredients and product types. Moreover possible entry pathways of the DBPs into the environment are investigated. The obtained results are finally complemented by an experimental validation via chemical analysis of simulated product applications.

WEPC23

Microbiological analysis and genotoxicity assessment of sewage effluents treated by advanced oxidation processes

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Among the processes for effluent treatment in Wastewater Treatment Plants (WWTP), advanced oxidation disinfection, such as ozonation (O_3) and ultraviolet radiation in conjunction with the hydrogen peroxide ($UV+H_2O_2$), can be highlighted. These processes generate hydroxyl radicals (OH·), which promote the oxidation of the pollutants and the disinfection of effluents. As the oxidative processes can generate toxic compounds and metabolites, bioassays are a crucial tool to evaluate the elimination of the toxicity, before these effluents are reused or discarded in hydric bodies. The application of new technologies to the effluent treatment requires a validation of the efficacy of the process to avoid any impairment of the ecosystem associated with the receiving water body. Thus, this study aimed to asses the genotoxic potential of sewage effluent samples, before and after the disinfection treatment by advanced oxidative processes, by the micronucleus (MN) test on HepG2/C3A cells, as well as the microbiological assessment of these effluents. Samples from a WWTP in Limeira (São Paulo State-Brazil) were collected after the conventional treatment process. The samples were exposed to treatments with ozone (O_3) and $UV+H_2O_2$ for periods of 20 (T1) and 40 (T2) minutes. The results showed a significant rate of MN induced by the effluent before and after the treatment with $UV+H_2O_2$ (T1). Regarding the nuclear buds, significant results were observed for the treatments $UV+H_2O_2$ (T1 and T2). The presence of multiple nuclear alteration was significant for the treatment $UV+H_2O_2$ (T1) and, when considered all the previous alterations combined (MN, nuclear bud and multiple alterations), the treatments $UV+H_2O_2$ (T1 and T2) were the ones that showed significant results. In relation to the microbiological characterization, the effluent before the treatment showed 8 bacteria genders. After the treatment with O_3T1 and O_3T2, 7 and 5 microbial genders were observed, respectively. After the treatment with $UV+H_2O_2$ (T1), only one gender was detected, which one was eliminated after $UV+H_2O_2$ (T2). Therefore, this study revealed that the treatment performed with $UV+H_2O_2$ induced more genotoxic damages to the cells, independently of the disinfection period assessed. In contrast, it was more efficient to eliminate the microorganisms. So, we must consider not only the disinfection power of the treatments, but the possible negative impacts that they may cause to the environment.

WEPC24

Effect-directed analysis for the determination of genotoxic products formed during ozonation of wastewater containing significant industrial inputs

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Organic micropollutants (MPs) such as pharmaceuticals and biocides cause undesired effects in the aquatic environment when present above certain concentrations. Discharges from wastewater treatment plants (WWTPs) are one of the major sources of introduction of MPs into watercourses. While conventional WWTPs are efficient at the removal of organic material and nutrients, their removal of MPs is insufficient. As a result, upgrading technologies in WWTPs are crucial to minimize the discharge of MPs into watercourses. Ozonation is one of the measures to remove a broad range of MPs from wastewater (WW). In Switzerland, a new water protection act, which entered into force in January 2016, requires the reduction of MP discharges from WW. To comply with this requirement, several Swiss WWTPs have been (or are being) upgraded with ozonation treatments. During ozonation, most MPs react either directly with ozone or with hydroxyl radicals formed from ozone decomposition processes, leading to the formation of ozonation transformation products (OTPs) and byproducts (OBPs), which may also be toxic. Although some of the formed OTPs and OBPs are removed in biological post-treatments, there are still concerns about those that persist. Additionally, the impact of the presence of significant industrial WW discharges into municipal WWTPs remains an issue of concern, since previous and recent own investigations highlighted increased toxicities during ozonation in such WWTPs in some cases. The present study aims at optimizing and implementing a diagnostic bioanalytical strategy integrating toxicological and chemical analyses based on effect-directed analysis (EDA). This approach combines *in vitro* genotoxicity assays, fractionation, and high-resolution mass spectrometry (HRMS) for the detection and identification of genotoxic compounds formed during the ozone treatment of wastewater rich in industrial inputs. Based on the obtained results, thorough investigations of the formation mechanisms of genotoxic compounds will be conducted to characterize their precursors and understand the conditions which affect their formation.

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